University of Alberta

Conservation Genetics of North American Fishers, Martens, and Wolverines

by

Christopher Jonathan Kyle ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Systematics and Evolution

Department of Biological Sciences

Edmonton, Alberta

Fall 2002

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Conservation Genetics of North American Fishers, Martens, and Wolverines submitted by Christopher Jonathan Kyle in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Systematics and Evolution.

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Abstract

Molecular methods were used to investigate and compare determinants of genetic variation and population structure in four closely related terrestrial mustelids. Genetic structure was influenced by several life-history characteristics, such as dispersal capability and habitat specificity, but results suggest that historical demographic trends have also contributed to the contemporary levels of genetic structure observed for these mustelids. Wolverines (*Gulo gulo*), with their capacity to disperse vast distances in a short period of time and relative lack of habitat specificity, display low levels of genetic structure in northern regions. However, populations were more structured at the southern and eastern periphery of their North American range, a result that may be attributed to historical anthropogenic pressures. Fishers (*Martes pennanti*) and European pine martens (*M. martes*), with more limited dispersal potential and higher habitat specificity than wolverines, were strongly structured throughout their respective distributions. However, trapping, poisoning, and habitat destruction caused significant population declines and contracting distributions in the early 1900's for these species, and this may also have resulted in genetically distinctive populations. Thus, historical demographic trends from anthropogenic sources may be responsible for increased genetic structure in peripheral wolverine populations, fishers, and European pine martens. In contrast, weak isolation by distance was observed between all mainland populations of American pine marten (*M. americana*) populations in Canada. If this species is indeed a habitat specialist with limited dispersal capabilities, as suggested, then there would be a

presumed impediment to gene flow between regions. Hence, the lack of genetic structure observed for Canadian martens could be explained by relatively large effective population sizes that have reduced the effect of genetic drift and maintained relatively homogenous genotypic frequencies among populations. Alternatively, martens may not be as habitat specific as previously thought, and these results could reflect frequent dispersal events across a relatively unfragmented landscape in Canada. Elucidating the population genetic structure of these four species has provided further insight into their ecology and potential future viability, by identifying isolated populations with decreased genetic variation that may be more susceptible to local extirpation.

Acknowledgements

I would like to begin by thanking my advisor, Curtis Strobeck, who has provided me with the opportunity to conduct the various studies contained herein and for the resources he has made available to me. I was never constrained in the types of projects I wanted to undertake and as such I was exposed to an environment that has allowed me to explore my own potential for independent research. I would also like to thank my committee members, David Hik and Ian Stirling, for their help in guiding my research along the way. NSERC and Parks Canada grants to Curtis Strobeck are responsible for funding all of my research.

To my labmates I can only say that being surrounded by such a group of interesting and intelligent people has made my tenure at this university enjoyable and memorable. Your input, support, and patience has been greatly appreciated.

Obviously, none of this work would be possible without the support of the numerous individuals and institutions to help collect the thousands of samples from across North America, Europe, and Russia that have been included in this thesis. Please refer to the acknowledgement section of each chapter where I thank everyone who was so generous with their time and effort in helping me with my research.

On a personal level, the value of the support and encouragement from my family and Jennifer McLean can not be measured. In many ways, I am indebted to you all.

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Chapter 1

General Introduction

Carnivores are integral to the proper functioning of ecosystems, yet mid-sized to large carnivores have historically been persecuted wherever they may come into contact with humans (e.g. *Ursus arctos, Canis lupus, Felis concolor*). Persecution comes in several forms including predator control programs initiated when conflicts arise with animal husbandry when carnivores prey upon livestock or game species; human development compromising many regions of suitable carnivore habitat; and direct elimination through fur harvest and trophy hunting. Globally, these factors have resulted in many carnivores presently inhabiting only a fraction of their historic ranges, and where they persist, many populations have been drastically reduced in size (Woodroffe 2001). The steady retraction of range has potentially led to more isolated, and sometimes completely fragmented, populations on the periphery of a continuous core of populations (e.g. Yellowstone brown bears, Paetkau et al. 1998). These populations often have smaller effective population sizes, and decreased levels of genetic variation due to genetic drift; a problem that is compounded by carnivores often having inherently low densities as top predators. The long-term viability of such populations may be compromised (Gilpin and Soule 1986, Soule and Mills 1998), as suggested for several carnivore species (lions, Gilbert et al. 1991; Florida panther, Roelke et al. 1993; Northern elephant seals, Hoelzel et al. 1993; and cheetahs, O'Brien 1998).

Using recent genetic techniques it has been possible to obtain further insight into not only the genetic variation and structure of populations, but also ecological characteristics of many carnivores that are difficult and expensive to observe directly using traditional techniques (e.g. wolves, Roy et al. 1994; polar bears, Paetkau et al. 1995; and brown bears, Paetkau et al. 1998). Genetic variation and structure within a species is reflective of ecological life-history traits such as dispersal, fecundity, and density. A species' sensitivity to topographic or anthropogenic sources of fragmentation can result in genetic structure between populations, and some studies have shown behavioural characteristics may also have a substantive role in how populations are genetically subdivided (e.g. Carmichael et al. 2001).

Mustelids are no exception to the general demographic and historical distribution trends observed for many carnivores, and as such these species have been recognized by several conservation committees (e.g. COSEWIC, CITES, IUCN, WWF) as species of special conservation concern. Mustelids, however, are relatively enigmatic species that are difficult to observe in the wild. This has resulted in a general paucity of ecological data for many species of this family relative to other carnivores, and a concomitant lack of genetic studies.

The primary goal of this thesis is to investigate genetic variation and structure in various mustelid species to contribute to the understanding of mustelid ecology across Canada, and to provide more insight into the underlying factors influencing the structuring of these species. By investigating several closely related mustelid species, including the wolverine (*Gulo gulo*), fisher (*Martes pennanti*), and American pine marten (*M. americana*), patterns across species sampled from a similar range can be revealed. These trends may more clearly depict which factors, including: topographic barriers, behavioural and life-history traits, or historical demographics, have the greatest influence on gene flow in these species. These studies also provide an additional basis with which to assess conservation strategies for each species by identifying isolated populations, potentially influencing current trapping strategies and quotas for particular regions and populations where mustelids are still harvested for their fur.

Wolverine (*Gulo gulo*)

Wolverines (*Gulo gulo*) are the largest terrestrial mustelids with adult male wolverines weighing between 12-18 kg. As with many mustelid species, sexual dimorphism is pronounced; adult females weighing 30-40% less than males. The species thought to be polygamous, with males and females reaching sexual maturity at two years of age. Males can stay reproductive for up to 14 years as compared to females which stop breeding 1-2 years earlier (Blomqvist 1995). Females produce on average 2 to 3 kits per year (Banci and Harestad 1988), but juvenile mortality rates can be very high. The life expectancy of this animal is approximately 8 to 10 years and up to 17 in captivity (Blomqvist 1995). The main cause of mortality in this species is by humans (trapping), followed by starvation and predation by other carnivores (*Felis concolor* and *Canis lupis*) as it attempts to scavenge their kills (Boles 1977, Magoun 1985, Banci 1994, Copeland 1996).

Presently, wolverines have a circumpolar distribution and are found in boreal forest, tundra, and old growth forests. The Old World wolverine is found in northern Eurasia from Scandinavia eastward to Siberia and Asia (Wilson and Reeder 1993). The New World wolverine was once found across North America. In the United States, it ranged from Maine to Washington State and southward along the Rocky Mountain ranges to Arizona and New Mexico (Hash 1987). Wolverines exist continuously on mainland Alaska (LeResche and Hinman 1973), but in the contiguous United States it is now restricted to the northwest, most likely as a result of peninsular extensions from Canadian populations (Banci 1994, Hash 1987). In Canada, wolverines were once found across the country from British Columbia to western New Brunswick including the Canadian Territories (Banfield 1987, Banci 1994). Currently, eastern populations are thought to be extirpated with no reliable sightings in Quebec or Labrador. The last wolverine caught in Labrador was in 1979 (M. Huot, pers. comm.). This species is very rare across the Canadian prairies, found mostly in northern regions (Van Zyll de Jong 1975). In Alberta, wolverines once existed throughout all coniferous forests in the province (Soper 1964), but they are now restricted to the northern regions and along the British Columbia border (Peterson 1997; see Fig. 1-1).

The home range of male wolverines is larger than that of females, often encompassing 2 to 3 female home ranges. Female home ranges are reduced even more when they are with kits (Banci 1987). Home ranges also seem to vary seasonally (Hatler 1989). Seven separate studies have reported home range sizes of wolverines in North America. Krebs (1995) reported values ranging from 48 to 778 km^2 in British Columbia. The other Canadian study by Banci and Harestad (1990) in the Yukon territory revealed male home range sizes to vary between 209 to 269 km^2 and 76 to 269 km^2 for females. Several Alaskan studies have also taken place and reported the following average values for male home ranges: 535 km^2 (Whitman et al. 1986), 637 km^2 (Gardner 1985) and 666 km^2 (Magoun 1985). The only female home range reported for Alaska was 104 km^2 (Magoun 1985). In Montana, Hornocker and Hash (1981) reported the home range of males to average 422 km^2, whereas females had a home range averaging 388 km^2. In Idaho, Copeland (1996) found home range sizes to be the largest, averaging 1522 km^2. The variance in these home range estimates may be explained by habitat and food availability (Gardner 1985).

Wolverine density is also highly variable, with values ranging from 1.25 to 25 animals per 1000 km^2, of "suitable" habitat, being reported (Banci 1987). Golden et al. (1993) recorded Alaskan wolverine densities to be about 5.2 animals per 1000 km^2 whereas, in Montana (Hornocker and Hash 1981) studies reported values of 15.4 animals per 1000 km^2. In the Yukon, Banci and Harestad (1990) reported a density of 5.6 animals per 1000 km^2.

The numbers of wolverines seem to be decreasing globally with human encroachment on remote habitats. They are, potentially, susceptible to overharvesting due to their inherent low densities and low reproductive rates (Banci 1994). These animals seem to thrive in areas which are undisturbed and this may play a crucial role in their conservation. Furthermore, the connectivity of these areas might also be an important factor affecting the levels of genetic variation in these populations. The aforementioned factors have led to wolverines being listed from vulnerable to endangered across most of its global distribution. In the United States, the Biodiversity Legal Foundation (1994) attempted to have *Gulo gulo* listed as an endangered species in contiguous United States. This petition, however, was denied by the United States Fish and Wildlife Service (U.S. Department of Interior, Fish and Wildlife Service 1995 (USFWS)) due to a lack of information suggesting that this status be granted. This is now the subject of an ongoing lawsuit against the USFWS, and new petitions have since been put forth (see www.thewolverinefoundation.com). In Canada, the eastern population of wolverines has been listed as endangered by COSEWIC (2001) and vulnerable across the rest of its Canadian range. Both Alberta and British Columbia have blue listed (vulnerable species status) *Gulo gulo*. Old World wolverines are considered vulnerable to rare in Norway, vulnerable in Sweden, Russia and Estonia, and endangered in Finland (Landa and Skogland 1995).

Fisher (*Martes pennanti*)

Fishers *(Martes pennanti)* are the largest member of the *Martes* genus and have no taxonomic equivalents in Europe and Asia as do American pine martens and wolverines. Adult male fishers weigh between 3.5 and 5.5 kg and are between 90 and 120 cm in length. Adult females weight approximately 50% less than males and are approximately 25% shorter. Fishers inhabit mainly dense conifer forests, especially spruce-fir habitats (Thomasma et al. 1994), avoiding areas with little canopy cover (Powell 1993). Sexual maturity is reached between the ages of 1 and 2 years and they may to live up to ten years in the wild and in captivity. Litters average three young, but litters of up to six have been observed (Nowak 1991). Fishers are also thought to be the only specialized hunter of *Erethizon dorsatum* (porcupines) and have been introduced as a "control" species for porcupines in Alberta, Manitoba, Montana, Oregon, and Washington (Powell 1993). Juvenile dispersal is relatively limited in this species with males and females dispersing an average 11km from their natal range with observations of dispersion up to 20km (Arthur et al. 1993).

Fishers were once found throughout the northern forest up to about 60 degrees north latitude and south along the Appalachian and Pacific coast mountains (Graham and Graham 1994; see Fig. 1-2). Since this time, with extensive habitat destruction from logging and trapping, their distribution has been substantially reduced (Powell 1993). For this reason, fishers have been the subject of several reintroduction programs where they were thought to have been extirpated.

In harvested regions the home range size reported for male fishers varies from 17 to 79 km^2 with a mean value of 40 km^2. For females the home range size varies from 4 to 32 km^2 with a mean value of 15 km^2 (Powell and Zielinski 1994). These values seem to vary with habitat type, season, and trapping (Garant and Crete 1997).

Reported fisher densities range from one animal per 2.6 to 20.0 km^2 (Powell and Zielinski 1994) and appears to vary seasonally. A study by Arthur et al. (1989) in Maine reported winter densities to be from one animal per 8.3 to 20.0 km^2 in winter and one animal per 2.8 to 10.5 km^2 in summer. Garant and Crete (1997) found densities of 9.2 km^2 and 5.4 km^2 for males and females, respectively in an untrapped area in Quebec. Fisher densities are quite variable, however, and seem to follow the cyclical patterns of population increases of prey such as snowshoe hares (Bulmer 1974).

In the United States, fishers are considered protected species in Oregon, Utah, Washington, and Wyoming while California and Idaho have closed their trapping seasons. Fishers are being considered for "threatened" species status in Washington (Powell and Zielinski 1994). In Canada, this species has been afforded no special species status with nearly all trapping seasons remain open across the provinces and territories.

American Pine Marten (*Martes americana*)

The American Pine marten (*Martes americana*) is probably the most important North American furbearer in terms of quantity. Adult male martens weigh between 0.5 and 1.4 kg and are between 50 and 68 cm long. Females of this species are between 20 and 40% smaller than males. Martens are thought to exist in a very narrow range of habitat types strongly associated with coniferous forests (Allen 1987). Most females are sexually mature after 24 months (Strickland et al. 1982) with mean litter sizes being 2.85 and ranging from 1 to 5 young (Strickland and Douglas 1987). Breeding can occur until approximately 12 years of age (Mead 1994) with a life expectancy of 15 years both in the wild and in captivity (Strickland and Douglas 1987). Trapping is thought to be the highest source of mortality in harvested populations, accounting for up to 90% of all deaths (Hodgman et al. 1993).

Martens are found in all temperate to arctic zones spanning the continent including many offshore islands (Hall 1981), however the majority of the distribution is in the boreal and taiga zones of Canada and Alaska (Buskirk and Ruggiero 1994; see Fig. 1-3). Population distributions roughly follow the distribution of coniferous tree species (Hall 1981). This species can disperse up to 40-80 km from its natal home-range (Thompson and Colgan 1987).

The average home range of an American pine marten has been reported to range from 0.8 km^2 in Montana (Burnett 1981) to 15.7 km^2 in Minnesota (Mech and Rogers 1977). Female home ranges are said to be 53% smaller than those of males (Buskirk and Ruggiero 1994; Buskirk and MacDonald 1989). As expected, home ranges vary according to prey abundance and habitat as found by Thompson and Colgan (1987). In a study by Soutiere (1979) comparing undisturbed and clear cut forests in Maine, it was shown that home ranges were 67% larger in clear cut areas.

Reported densities of martens range from 0.4 to 2.4 animals per km^2 which are the values reported by Thompson and Colgan (1987) for Ontario martens in times of prey abundance and scarcity. Values of 0.6 animals per km^2 have been recorded in the Yukon (Archibald and Jessup 1984, Francis and Stephenson 1972). In comparing an undisturbed forest and a clear-cut forest in Maine, Soutiere (1979) found densities of 1.2 and 0.4 animals per km^2, respectively.

In Canada the only marten population given endangered status by COSEWIC (2001) is the subspecies, *M. a. atrata,* in Newfoundland, although other populations in Nova Scotia (including Cape Breton), Prince Edward Island are thought to be endangered or extinct (Thompson 1991). The decline of this species has been attributed to trapping and logging practices leading to the loss of suitable marten habitat (Thompson 1991). In the United States, although they are not officially listed as endangered, martens may not be legally trapped in California, Nevada, New Mexico, South Dakota, and Utah. Furthermore, in Utah, martens are listed as a protected species and endangered (Group II) in New Mexico (Buskirk and Ruggiero 1994).

European Pine Martens (*Martes martes*)

The European pine marten (*M. martes*) is allopatric and often considered ecologically equivalent to the American pine marten. This species is thought to be a habitat specialist, preferring mesic mixed forests with overhead cover (Clevenger 1994). European pine martens sexually mature at 2yrs, have 2-8 young, and usually live to 7 years of age, but have been observed to live up to 17 years in captivity (Nowak 1991). The main sources of mortality in this species are hunting, poisoning, roads, and predation from foxes (Bright and Smithson 2001).

European pine martens are found in western Europe to western Siberia including the islands of Ireland and Great Britain (Nowak 1991). Until the 19th century martens were found throughout Britain, but were thought to be restricted to northwest Scotland by 1926. Protective legistlation has since resulted in an increasing range in Scotland, however, indications of their presence in England and Wales remain unclear.

The home range of this species normally ranges from 10-25 km^2 in males to 5-15 km^2 in females, but Zalewski et al. (1995) found much smaller home ranges in Poland (2.23 km^2 for males, 1.49 km^2 for females). Densities range from 1 per km^2 on average, but can be as low as 1 per 10 km^2.

Martens were excessively trapped and greatly reduced in number in 20th century in Britain, with two main periods of overharvesting recorded in Scandinavia in the 1500-1600s and early 1900s (Helldin 2000). The precipitous decline in numbers was also compounded by eradication efforts from poisoning. In Great Britain martens are protected by the Wildlife and Countryside Act (1981).

Life History and Genetic Structure

Fecundity, generation time, and mating systems

Fecundity, generation time and mating system can all have an effect on the levels of genetic variation and structure observed for a species (Avise 1994). Short generation times and high fecundity can led to large effective population sizes that harbour much genetic variation and maintain homogeneous genotypic frequencies. Wolverines, martens and fishers are all thought to be nearly equivalent with respect to these life history traits. All three species are thought to be polygamous, mature at about 2 years of age (Strickland et al. 1982, Nowak 1991, Banci 1994), have between 2-3 young (Strickland and Douglas 1982, Powell 1993, Banci 1994), and have life expectancies of around 8-10 years (Nowak 1991, Blomqvist 1995). Hence, no net effect was expected among the three species.

Influence of habitat specificity

Wolverines are considered habitat generalists, existing in habitats ranging from boreal forest to tundra (Banci 1994). It was, therefore hypothesized that this trait would result in little genetic structure for this species (see Chapters 2 and 3). A high degree of genetic structure was expected in both martens and fishers (see Chapters 4, 5, and 6), however, from the suggestion that they are strongly associated with old-growth forests and avoid regions with little canopy cover (see Powell and Zielinski 1994; Buskirk and Ruggiero 1994); habitats that have largely been removed from much of southern Canada and Europe (Chapter 7).

Influence of dispersal

In order to investigate the influence of dispersal potential on the genetic structure of these species, wolverines, martens, and fishers were sampled from across much of their current North American distributions (Chapters 2, 3, 4, 5, and 6). Given the dispersal potential of wolverines (>300km Magoun 1985, Gardner 1985, Copeland 1996), prompting the theory that this species may exist as a panmictic unit in North America (see Banci 1994), little genetic structure was expected. Fishers and martens have more limited dispersal abilities, with fishers only observed to move up to 20km (Arthur et al. 1993) and martens between 40-80km (Thompson and Colgan 1987). Hence, more genetic structure may be observed in these species relative to wolverines. Home-range sizes are also often correlated to dispersal potential, and therefore, genetic structure. The home-range of wolverines range from as small as 48 km^2 (Krebs 1995) to 1522 km^2 (Copeland 1996), in fishers home-ranges are often found to be around 40 km^2 (Powell and Zielinksi 1994), whereas martens have home-ranges of 0.8 km^2 to 15.7 km^2 (Mech and Rogers 1977). In all three species the home-range of females is approximately 50% smaller than that for males. From these observations, more structure would be expected in fishers and martens relative to wolverines.

Density

Populations size and density of species are interrelated. Higher densities often lead to less structured populations where genotypic frequencies are less likely to be influenced by the process of genetic drift. Wolverines are very scarce, with densities ranging from one animal per 40 – 670 km^2 (see Banci 1994), fishers are found to have densities ranging from one animal per 2.6 – 20 km^2 (see Powell and Zielinksi 1994), and martens were found in the highest numbers, ranging from one animal per 0.4 – 2.5 km^2 (see Buskirk and Ruggiero 1994). These observations would suggest that the most structure might be observed in martens, fishers would have an intermediate level, and wolverines would be the least structured of these species (see Chapters 2, 3, 4, 5, and 6).

Historical demographics and the influence of anthropogenetic pressures on population genetic structure

When species are narrowly distributed or exist in a fragmented landscape more genetic structure is expected due to the processes of genetic drift (Avise 1994). Wolverines and martens were historically broadly distributed throughout Canada (with the exception of the prairies), while fishers are normally only found south of 60° latitude (Banci 1994, Banfield 1987, Graham and Graham 1994, Hall 1981, Buskirk and Ruggiero 1994). These respective distributions include several offshore islands where populations might be expected to be genetically distinctive based on their insularity. It is the contracting range of these species in the late 1800's and early 1900's, however, that may have an important role in how mainland populations are structured. These contractions in range may have left remnant populations that have become most structured through drift (see Chapters 2, 3, 4, 5 and 6).

Anthropogenic influences likely explain the contracting distribution of these species. Wolverines were subject to predator control programmes in North America (mainly targeted at wolves, Banci 1994) and their numbers were significantly reduced in the early 1900's, especially in the lower 48 states where they were thought to have been nearly extirpated (Davis 1939, Newby and Wright 1955, Newby and McDougal 1963). Thus, any remnant wolverine populations, like that observed for Yellowstone brown bears (Paetkau et al. 1998), may be genetically distinct from other populations. In Chapter 2, populations from the southern (Idaho, Revelstoke) and eastern (Manitoba) periphery of the current distribution of wolverines were examined. Chapter 3, which expands upon the work in chapter 2, includes more samples from regions at the periphery of the current distribution of wolverines (Wyoming and Ontario), Russia, and more northern populations. The samples from Russia, separated from North America for >10, 000 years, would provide a baseline from which to compare the genetic distinctiveness of populations within North America.

Fishers were eliminated from much of their historical range in the early 1900's (Powell and Zielinski 1994). As such, a significant level of structure was expected between extant populations of this species (Chapter 4). Samples were also obtained from regions where fisher re-introductions had taken place. It was hypothesized that these regions, that have effectively gone through severe population bottlenecks, would have decreased levels of genetic variation through genetic drift relative to indigenous populations.

In martens, given the suspected level of fragmentation of suitable marten habitat in southern reaches of the Canadian provinces relative to the Canadian territories from anthropogenic influences, it was expected that much less structure would be observed in northern regions than southern. In Chapter 5, martens were obtained from several regions in the Canadian Territories. In Chapter 6, samples were obtained from across Canada, excluding most martime provinces, but including animals from Newfoundland island where they are considered a separate subspecies (*M. a. atrata*). More structure was expected in the Canadian provinces, relative to that found in the northern regions of Canada, given the level of habitat homogeneity in northern regions and the potentially fragmented nature of suitable marten habitat in much of southern Canada.

European pine martens have existed in an environment that has been heavily modified for centuries. Few previous genetic studies have taken place on this species. Davison et al. (2001) used mtDNA to investigate the genetic structure of European pine martens sampled from northwestern Europe, including Scotland, Ireland, and England. They found relatively little genetic variation among mainland populations, but significant structure between regions. These same samples were obtained to investigate the genetic structure of these species using microsatellites (Chapter 7). It was expected that a relatively high level of structure would be observed given previous results with mtDNA, and the fragmented habit in which this species still persists. These data would also be used as a comparison to findings for North American species, and in particular, American pine martens, thought to be ecologically equivalent to European pine martens.

Trends Among Species

In the concluding chapter (Chapter 8), findings for each of the species under investigation are summarized, relative to initial predictions. Following this, trends observed among the species across their North American range will be examined. Comparisons will also be made between findings for mustelids and other mid to large sized North American carnivores. Finally, the future of mustelid genetic research will be discussed.

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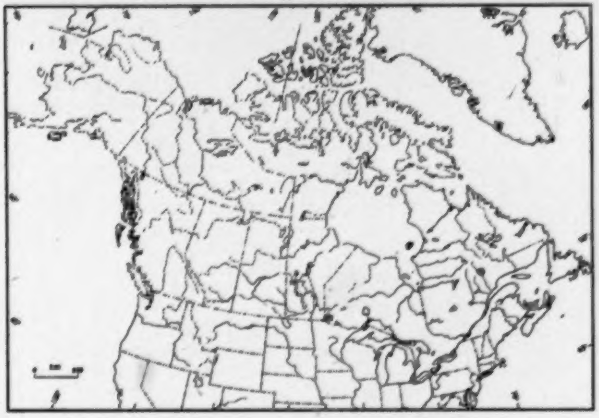
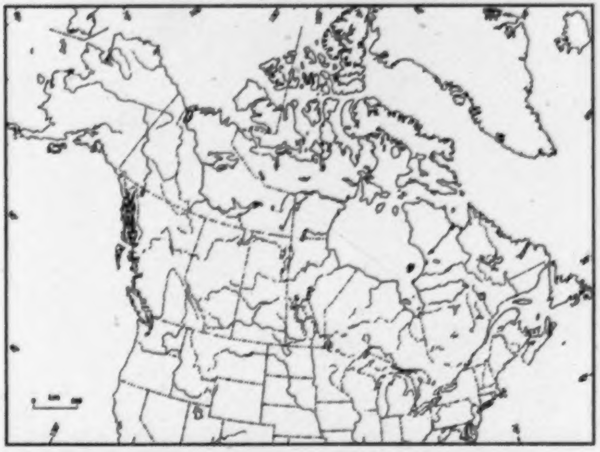
Figure 1-1. Approximate current North American distribution wolverines, fishers and martens (modified from Banfield 1987).

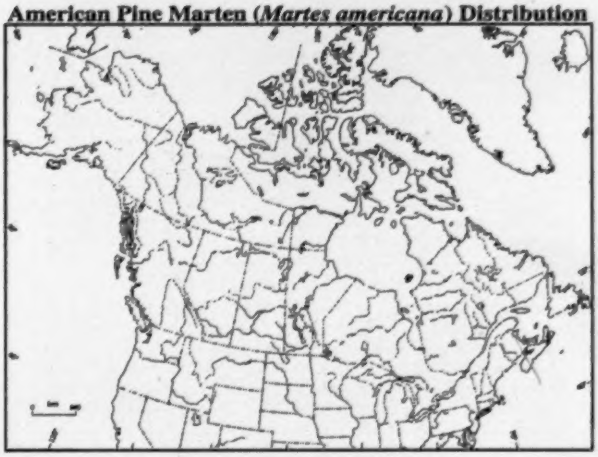
Wolverine (*Gulo gulo*) Distribution



Fisher (*Martes pennanti*) Distribution



American Pine Marten (*Martes americana*) Distribution



Chapter 2

Genetic structure of North American wolverine (*Gulo gulo*) populations

Introduction

Wolverines (*Gulo gulo*) are the largest terrestrial member of the family Mustelidae (the weasel family). This species has a circumpolar distribution and is found in tundra, taiga and forest zones of North America and Eurasia (Wilson 1982). In North America, prior to human settlement, wolverines were distributed across Canada and Alaska with projections southward into the conterminous US through montane regions as far as New Mexico and Arizona (Hash 1987). Currently, wolverines in Canada west of Hudson's Bay are found in the northern regions of the prairie provinces, in Alberta's western national parks, and throughout most of British Columbia and the Canadian Territories (Banci 1994). East of Hudson's Bay, wolverines are exceedingly rare and are listed as endangered by COSEWIC (1998). In the conterminous US the range of wolverines has steadily retracted since the 1840s, with animals persisting in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California (Hash 1987). The contracting range of wolverines in North America is the result of habitat loss, overharvest and other anthropogenic factors, which together have led to a decline in the numbers of these animals (Wilson 1982; Banci 1994).

Wolverines are found at low density across their Holarctic distribution with estimates of one animal per 40–800 km^2 (see review by Banci 1994). This species also establishes large home-ranges (100–900 km^2) which seem to be maintained between years (Magoun 1985; Banci 1987) and which vary in size in relation to food abundance (Banci 1994). Males typically maintain larger home-ranges than females and may overlap the home-ranges of several females (Banci 1994). Wolverines are reproductive from 2 years of age onwards, although the fecundity of females is thought to decrease after the age of 6 (Banci and Harestad 1988). Not all females become pregnant in a given year (53–92% in the Yukon; Banci and Harestad 1988) depending on food availability. Even when females do give birth, kit mortality is thought to be high with estimates of $\approx$ 36%. These figures are considered to underestimate the true mortality rate (Banci 1994).

Most juvenile females exhibit natal area fidelity and establish home-ranges adjacent to their mothers (Magoun 1985), although some females have been observed to disperse far beyond their natal range. Subadult males typically disperse 30–100 km from their natal home-range (Gardner 1985; Magoun 1985), although several movements further than this have been reported. Gardner et al. (1986) reported a 378-km straight-line movement of a 2-year-old from southcentral Alaska to the Yukon Territory in a 7-month

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***A version of this chapter has been published. Kyle, C.J. and C. Strobeck, Molecular Ecology 10: 337-347, 2001.**

period. A yearling female was reported by Magoun (1985) to have dispersed 300 km in a 5-month period. It should be noted, however, that some rare long-distance movements in this species do not seem to be linked to dispersal, but are simply temporary forays from their homerange (Banci 1994). It is believed that half to one-third of all dispersing individuals die, either by starvation, harvest or predation (Krott 1982). The movement of wolverines does not seem to be influenced by the presence of lakes, rivers, mountain ranges or other topographical features (Hornocker and Hash 1981; Banci 1987). Only human development and major access routes are thought to function as barriers to dispersal for this species. Furthermore, human activity may influence kit survival, thereby limiting the expansion of wolverine populations (Banci 1994).

With increasing concern about the status of this forest carnivore and how it might be managed effectively, it is necessary to determine the genetic distinctiveness of wolverine populations and the levels of gene flow between geographical areas. Population genetic data may also be used to determine whether harvested populations are replaced solely from within or if replacement from other populations plays an important role in maintaining a population's numbers.

Wolverines, where they persist north of the 38th parallel in North America, are currently considered to be a continuous breeding group, however, the extent of movement between the larger Canadian populations and remnant conterminous US populations is unknown (Banci 1994). Recent work by Edelman and Copeland (1999) suggests that movement between Idaho and Oregon populations may be restricted due to a lack of suitable habitat between them. These findings may be reflected in the population genetic structure of wolverine populations in this area. Another recent study by Wilson et al. (2000) evaluated the genetic variability of wolverines in the Northwest Territories, Canada using both mitochondrial DNA (mtDNA) and allozyme markers. A high degree of population structure was found using mtDNA ($F_{ST} = 0.536$), although much less structure was observed among the sampled regions using nuclear allozyme markers ($F_{ST} = 0.076$). These results are not surprising given this species' high potential for dispersal, chiefly male dispersal, and female fidelity to natal areas. These findings, however, were based on small sample sizes (*n* = 3, 3, 3, 12 and 20 for each of the five studied 'populations', respectively) and limited genetic variation which the authors admit pose considerable constraints on the conclusions which can be drawn from the study.

The first goal of this study was to elucidate the population genetic structure of wolverines across much of their North American distribution, which to date is largely unknown. The second goal was to determine what underlying factors influence the population genetic structure of this species. We hypothesize that the genetic structuring of wolverine populations will be higher in the southern regions of this species' range, where anthropogenic factors potentially act as barriers to dispersal, than those populations found in undisturbed northern habitats.

To detect genetic differentiation among wolverine populations, high resolution (fast evolving) neutral genetic markers are preferred. Previous studies have found little or no genetic variation in wolverines using other markers (haemoglobin, Seal 1969; lactate dehydrogenase, LeDoux and Kenyon 1973; 337 bp of mtDNA sequence and five allozyme markers, Wilson et al. 2000). For this reason, we have chosen to use hypervariable microsatellite loci. These polymorphic, tandem repeats of DNA have proven useful in other studies of mammalian species with high vagility, such as the polar bear (*Ursus maritimus*; Paetkau et al. 1995,1999) and the North American brown bear (*Ursus arctos*; Paetkau *et al.* 1998). Microsatellites identified clear genetic differentiation between populations of these animals despite the long-range movements known to occur in these species, and the fact that little variation was detected using other methods.

Materials and methods

Sample collection

Twelve polymorphic loci were used to examine the population genetic structure of wolverines from 12 geographical regions. Bone samples were acquired from six Alaskan regions (all samples were provided by the University of Alaska, Fairbanks Museum and are sorted according to their quadrat system): Anchorage ($n = 36$); Arctic region, including samples from Arctic, Fort Yukon and Black River ($n = 47$); Nabesna ($n = 38$); Russian Mission region, including samples from Russian Mission and Holy Cross ($n = 34$); Noatak region, including samples from Noatak, Point Hope, Point Lay, Meade River, Survey Pass and Howard Pass ($n = 38$); and the Nome region, including samples from Solomon and Nome ($n = 38$). All samples from Alaska date from the late 1950s through to the mid-1960s. Tissue samples were obtained from two Nunavut territory regions (then part of the Northwest Territories): Kugluktuk ($n = 67$) and Bay Chimo ($n = 40$). Ear plug and hair samples were obtained from the Revelstoke ($n = 47$) and Williston Lake ($n = 37$) regions in British Columbia, eastern Manitoba ($n = 28$) and central Idaho ($n = 14$) (Fig. 2-1). All samples, other than those collected from Alaska, are of recent origin (early 1990s).

Laboratory methods

DNA was extracted using a QIAamp® Tissue Extraction Kit (QIAGEN). The primers used to amplify the microsatellites were developed by: Davis and Strobeck (1998) in badgers (BA-1 and BA-4), martens (MA-3) and wolverines (GG-3, GG-4, GG-7, GG-14); by Duffy et al. (1998) in wolverines (Ggu 101, Ggu 216 and Ggu 234); by Flemming et al. (1999) in mink (Mvis-75); and by Dallas and Piertney (1998) in Eurasian otters (L-604). Amplification of DNA was performed as in Davis and Strobeck (1998). Note that non-variable microsatellite loci were not included in this study which may have the effect of biasing relative estimates of genetic variation between species.

DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. Analysis of DNA fragments was done using the programs Genescan™ analysis 2.02 and Genotyper® 2.0.

Data analysis

A G-test for heterogeneity (Sokal and Rohlf 1995) was performed for each of the sampled areas by making pairwise comparisons of allele distributions. Departures from Hardy–Weinburg equilibrium (HWE) were tested for each of the 12 loci as assessed by Genepop version 3.1d (Raymond and Rousset 1995) which uses a Markov chain method following the algorithm of Guo and Thompson (1992). This software was also used to evaluate genotypic disequilibrium among the loci used.

The relative genetic variation in each population was first assessed using allele frequency data from which the mean number of alleles, unbiased expected heterozygosity, H_E (formula as per Nei and Roychoudhury 1974), and unbiased overall probability of identity, P_{ID} (Paetkau et al. 1998) were determined. The genetic distances between the populations were estimated using two measurements: Nei's standard genetic distance, D_S (Nei 1972) which is calculated from genotype frequencies, and the genotype likelihood ratio distance, D_{LR} (Paetkau et al. 1997) which is calculated from genotype probabilities. These measures were both identified by Paetkau et al. (1997) to perform better than other measures of genetic distance. Both sets of genetic distance values were calculated by programs from the website, http://www.biology.ualberta.ca/jbrzusto/Doh/html, designed by John Brzustowski.

An unrooted neighbour-joining tree of the D_S values was created using Phylip3.572 (Felsenstein 1995). The geographical and genetic distance values were also entered into a two-way Mantel test (Mantel 1967) within the set of programs called the 'R' Package for mulitvariate analysis designed by Alain Vaudor (software package can be found on Pierre Legendre's website: http://www.fas.umontreal.ca/BIOL/legendre/) to determine the correlation between genetic distance and geographical proximity. This software package was also used to run a three-way Mantel test (as per Smouse et al. 1986). We used this method to determine whether the curves on the scatter plot of genetic distance against geographical distance had significantly different intercepts. Populations which had significantly different curves from the expectation of isolation by distance were taken to have some barrier to gene flow other than geographical distance present. Each population was tested by creating a matrix of ones and zeros for the presence or absence of a barrier, while controlling for the geographical distance. All geographical distances were calculated using the distgeo program, also found within the 'R' package, from approximate latitudes and longitudes for each region.

Pairwise F_{ST} estimates were obtained from the software package Genepop 3.1d (Raymond and Rousset 1995; as per Weir and Cockerham 1984). The assignment test (Paetkau et al. 1995), also found on the aforementioned web site, was run for all populations. This program determines both the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in the regions with which it is being compared. It then assigns each individual to the population in which that individual's genotype has the highest probability of occurring (see Waser and Strobeck 1998). Unlike other pairwise population statistics, the assignment test uses the information from each individual's genotype to determine how similar are the gene pools of the two populations. The test was always run with the option of replacing allele frequencies of 0 with 0.01.

The significance of the assignment test results was obtained using two types of randomization tests. In the first randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus in the combined gene pool (all populations) assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals assigned from population A to population B. When the proportion is < 0.05, there is evidence that significant heterogeneity exists between the genotype frequencies in populations A and B.

In the second randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus found in that population, assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals in population A assigned to population B. When this proportion is > 0.95 then there is a significant number of cross-assignments from A to B. This implies that some of the individuals in A must be in genotypic disequilibrium with population A and are therefore presumably immigrants into A from a population with allele frequencies different for those found in A. Ten thousand replicates were used to obtain the significance values for both randomization tests.

Results

Tests of disequilibrium

All 12 loci used in this study were tested for departures from HWE. After accounting for sample-wise error (as per the Dunn-Sidak method; Sokal and Rohlf 1995), four departures from HWE were found: locus GG-3 in the Williston Lake British Columbia (BC) region, locus BA-4 in the Manitoba region, and locus Ggu 234 in the Anchorage and Nabesna regions. All departures from HWE were found to be heterozygote deficits which may imply the presence of null alleles in these populations. Low copy number may also be responsible for the heterozygote deficit in the two Alaskan populations as the DNA was extracted from bone samples. As deviations from HWE were not found in other populations for these loci, they were retained for analyses. Genotypic disequilibrium was suggested for the following pairs of loci: GG-7 with GG-14 in the Manitoba population, GG-3 with BA-4 in the Bay Chimo region, and GG-4 with GG-14 in the Revelstoke region. As these genotypic disequilibria were not found in more than one population it is unlikely that any loci are physically linked. Hence, all loci were retained for analyses. It is possible that these disequilibria are the result of linkage disequilibrium in the founding population that has not yet dissipated or that a recent admixture of populations with differing gametic frequencies has occurred (Hartl and Clark 1989).

Heterogeneity of sampled regions

Twelve geographical areas were sampled in this study (Fig. 2-1). All sampled regions were found to have significantly ($P < 0.05$) different allele frequencies by a G-test for heterogeneity with the exception of the Russian Mission and Arctic regions and the comparison of the Russian Mission and Nabesna regions. Despite these findings we treated these three regions as heterogeneous populations because of their geographical separation from one another (»1000 km).

Genetic variation

The population with the highest mean number of alleles was in Arctic, Alaska (AK) with a value of 5.83, whereas Idaho had the lowest value of 2.83 (Table 2-1). H_E values ranged from 68% in Nabesna, AK to 42% in Idaho. P_{ID} values ranged from 1/36 000 000 000 in Nabesna, AK to 1/452 000 in Idaho (Table 2-1). The Revelstoke population also had a low P_{ID} value (1/377 000 000) relative to the other populations. The low estimates of the mean number of alleles in Idaho may be explained, in part, by the low sample size ($n = 14$) relative to the other populations ($n > 30$), although measures of heterozygosity are less affected by sample size.

Assignment test

The results from the genotype assignment test revealed little genotypic distinctiveness between the Alaskan regions sampled, as evidenced by the relatively low number of correctly assigned individuals (1/4 to 1/6). The one exception was Nome, in which 21/35 individuals were assigned to the region from which they were sampled (Table 2-2). Most cross-assignments in Alaskan regions went to other Alaskan regions. A relatively high number of cross-assignments also went to the Nunavut regions in which 5/36 Anchorage individuals were assigned to Kugluktuk and 6/47 Arctic individuals were assigned to Bay Chimo.

Slightly more structure was observed in the Nunavut populations, with 19/40 and 23/67 individuals assigned correctly to Bay Chimo and Kugluktuk, respectively. The majority of the cross-assignments in Kugluktuk went to Bay Chimo (20/67). In Bay Chimo, 7/40 individuals were crossassigned to both Kugluktuk and Russian Mission, AK.

Of the two British Columbian populations, Revelstoke demonstrated more population genetic structure than did Williston Lake. In Revelstoke, 35/47 individuals were assigned correctly, whereas the Williston Lake population had only 15/37 individuals assigned correctly to itself. In both British Columbian populations the cross-assignments were most likely to go to the other British Columbian region with 5/37 Williston Lake individuals assigned to Revelstoke and 4/47 individuals from Revelstoke assigned to Williston Lake.

Both Idaho and Manitoba were found to be highly structured populations with 14/14 individuals correctly assigned to Idaho and 23/28 individuals correctly assigned to Manitoba.

Pairwise F_{ST} *and genetic distance measures*

Pairwise F_{ST} values ranged from 0.0017 to 0.2157 (Table 2-3). The smallest F_{ST} values were found within Alaska (0.0017– 0.0251) and Nunavut (0.0070). There was also little structure between the Alaskan and Nunavut regions (pairwise F_{ST} ranged from 0.0135 to 0.0432). The highest pairwise F_{ST} estimates were found for the Revelstoke (0.0359–0.0720), Manitoba (0.0816–0.1205) and Idaho (0.1670–0.2157) populations.

Estimates of the likelihood ratio (D_{LR}) and Nei's standard (D_S) genetic distances paralleled the results from both the assignment test and pairwise F_{ST} estimates. D_{LR} values ranged from 0.24 to 8.87 and D_S values ranged from 0.03 to 0.36. The Alaskan populations were all found to be genetically similar using these measures with values of D_{LR} ranging from 0.24 to 0.68 and D_S values ranging from 0.03 to 0.05 with the exception of Nome. Nome was more genetically distinct from other Alaskan populations, with larger genetic distances relative to between the other regions. The two Nunavut populations were also genetically similar ($D_{LR} = 0.25$; $D_S = 0.03$). Pairwise comparisons of the Alaskan populations to the Nunavut populations yielded D_{LR} values ranging from 0.65 to 1.39 and D_S values ranging from 0.05 to 0.10, again suggesting little distinction between these regions. Similar to the findings of the assignment test and pairwise F_{ST} values, these genetic distance measures identified Idaho, Revelstoke and Manitoba as the most genetically distinct regions relative to all other regions sampled.

An unrooted neighbour-joining tree of the D_S values is illustrated in Fig. 2-2 which reveals the relationships of genetic distances to all populations. The length of the tree branches is relative to the genetic distances. The tree shows that the Alaskan populations cluster, the Nunavut populations cluster, the British Columbia populations do not cluster, but were closely associated, and the Manitoba and Idaho populations are each on their own branches.

The results from both genetic distance measures were entered into a two-way Mantel test with geographical distance. The results from these two tests suggest that both genetic distance measures are correlated to geographical distance (D_{LR}) vs. geographical distance $r = 0.63$ $P = 0.00004$; D_S vs. geographical distance $r = 0.63$ $P = 0.00003$). D_{LR} and D_S were also found to be highly correlated to one another ($r = 0.99$ $P = 0.00009$). The D_{LR} values were also entered into a three way Mantel test to test for the presence or absence of a barrier to gene flow while controlling for geographical distance. The results revealed that the Idaho population is highly correlated to a barrier to gene flow other than distance ($r = 0.90$ $P = 0.0002$). The Revelstoke population was not as highly associated with a barrier to gene flow other than distance, yet the result was still significant ($r = 0.46$ $P = 0.01$) (Fig. 2-3).

Discussion

This study has elucidated the population genetic structure of wolverine populations across a large part of their North American distribution. Our results suggest that there is little genetic structuring of populations in the northern regions of this species' range, as reflected by an assignment test, pairwise F_{ST} estimates, and two genetic distance measures, D_S and D_{LR}. The small amount of structure between northern regions was consistent with isolation by distance. Furthermore, we found the more southerly populations, in which anthropogenic factors are most pronounced, to be more genetically structured than those found in the north. These results reflect similar findings to another recent study of wolverines using allozymes (Wilson et al. 2000) which revealed little genetic structure between regions in the Northwest Territories using nuclear markers ($F_{ST} = 0.076$). A study by Paetkau et al. (1998) of North American brown bears (*Ursus arctos*) also reflects findings similar to those presented here. This is significant as both brown bears and wolverines have a similar distribution, density and potential for dispersal. In both these species, populations in the southern reaches of their range are more genetically distinct, and have less within-population variation, than those found in less disturbed northern habitats. Our results are also consistent with those found for another mustelid species, the North American pine marten (*Martes americana*). Marten populations sampled from the Yukon and Northwest Territories revealed little population genetic structure and much gene flow was evident across the entire northern sampling range (Kyle et al., 2000). We suggest that the level of genetic structuring in the southern reaches of this species' distribution is the result of low effective population sizes, restricted gene flow and potentially population fragmentation, whereas the low level of structure in the northern regions is consistent with high levels of gene flow and few barriers to dispersal.

Sampling in time and space

It is important to mention, as noted in Materials and methods, that all Alaskan samples collected in the late 1950s to mid-1960s were treated as a contemporary to samples collected in the 1990s. We made the assumption that 30–40 years difference (» 6–8 generations) between the collection times of the samples has had little effect on the genotype frequencies in these regions. There is little evidence to suggest that large demographic changes have taken place during the time separating the collection of these samples. We are unaware of any drastic population bottlenecks or large range expansions that may have greatly affected the genotype frequencies in these Alaskan regions during this time. We have also presented results suggesting that extensive gene flow exists among northern regions. With an extensive mixing of populations genotype frequencies would be less likely to change significantly over a period of six to eight generations. Despite our assumptions, we have no direct evidence that genotype frequencies have not changed in this time, therefore our findings may not reflect current levels of genetic variation and gene flow in these regions.

Lack of genetic structure in northern regions

Little structure was observed among the northern regions sampled in Alaska and Nunavut. Measures of F_{ST}, D_S and D_{LR} among the Alaskan regions revealed the smallest pairwise differences, supporting the conclusions from the assignment test that little structure exists between these regions despite large geographical distances separating them. To put these values into some perspective, although they are not directly comparable as different microsatellite markers were used, the smallest D_S value reported in brown bears by Paetkau et al. (1998) was between two adjacent Northwest Territory populations with a value of 0.053. The range of D_S genetic distances among wolverine populations in Alaska and Nunavut vary between 0.03 and 0.07 (Table 2-4).

The one Alaskan exception, which did reveal a slightly higher level of genetic structure relative to the other Alaskan regions, was Nome. This population had almost 2/3 of the animals sampled assigned to itself compared to only 1/6 to 1/4 of the individuals being correctly assigned in the other Alaskan regions (Table 2-2). It is unclear why this region would be more structured than other Alaskan regions, although the fact that Nome is found on a peninsular projection of Alaska may have somehow isolated this region.

The two Nunavut regions, Bay Chimo and Kugluktuk, are separated from each other by 400–500 km and reveal a similar pattern to that found in Alaska. There seems to be little genetic structuring between these northern regions, as reflected by the relatively high number of crossassignments shared between these two regions and the estimates of F_{ST}, D_{LR} and D_S (Tables 2-3 and 2-4).

There also seems to be little genetic structure between the Alaska and Nunavut populations. Of the two populations sampled in Nunavut many of the cross-assigned individuals went to Alaskan populations (Table 2-2). The genetic distance measures (Table 2-3) between Nunavut and Alaska are also relatively low, with Kugluktuk having slightly smaller pairwise estimates of genetic distance to the Alaskan populations than Bay Chimo, as follows from their geographical proximity to these regions (Fig. 2-1).

The lack of genetic structuring in the northern reaches of this species' range is most likely the result of extensive gene flow among these regions. This conclusion is supported by several wolverine life history characteristics: topographical features such as rivers, lakes and mountain ranges do not limit dispersal (Hornocker and Hash 1981; Banci 1987), subadult males are known to disperse large distances from their natal range to establish their own home ranges (Magoun 1985; Copeland, personal communication), and wolverines are capable of moving long distances with relative ease as documented by

Gardner (1985), Gardner et al. (1986), and Magoun (1985). The combination of these life history characteristics has most likely had the effect of genetically homogenizing wolverine populations in undisturbed habitats in which few barriers to dispersal exist. Only a few migrants per generation are capable of genetically homogenizing populations over time (Slatkin 1985).

An alternative to the aforementioned hypothesis is that the lack of structure observed in the northern reaches of this species' range may not represent extensive gene flow, but rather a relatively recent postglacial colonization of the north. However, wolverines were thought to exist in several refugia during the Wisconsin glaciation, both south of the ice sheets and in Beringia as revealed by fossil evidence (Bryant 1987). The current pattern of genetic structure therefore has most likely not been influenced by a rapid radiation of wolverines northward from a southern glacial refugium.

Without samples from the Yukon or Northwest Territories it is unclear whether the lack of genetic differentiation observed between northern regions in this study is true of all northern regions. Given that no large barriers to dispersal are present in these areas, and the life history traits of wolverines, we would expect a similar pattern of genetic structure to persist across all of these northern regions east of Hudson's Bay.

Despite the lack of genetic differentiation in the northern reaches of this species distribution we cannot say that all the animals are similar in adaptive traits. Adaptive traits can occur between populations showing little genetic structure at neutral genetic loci (Karhu et al. 1996).

Genetic structuring of western Canadian populations

Only two regions were sampled from western Canada, both from British Columbia. These regions, however, revealed very different levels of genetic structure. The Williston Lake population had a relatively high level of cross-assignments to all regions (only 15/37 individuals correctly assigned) and pairwise measures of F_{ST}, D_S and D_{LR} were comparable with those found for the Bay Chimo region in Nunavut (Tables 2-3 and 2-4). This central British Columbia region also seemed to be more closely associated with populations sampled in Nunavut and Alaska than the southern British Columbia population of Revelstoke with respect to these estimates (Tables 2-3 and 2-4).

The Revelstoke population was found to be relatively distinct from all other regions sampled, with 35/47 individuals correctly assigned to itself (Table 2-2). A plot of geographical vs. genetic distance (Fig. 2-3) also revealed that the D_{LR} measures in Revelstoke were higher than expected from the values in the northern regions. This difference was found to be significantly correlated to some barrier to gene flow by a three-way Mantel test ($r = 0.46$ $P = 0.01$).

The difference in genetic structuring between these two regions may potentially be attributed to differences in anthropogenic pressures. Both regions are intensely forested, yet the Revelstoke region also has major transportation corridors present (Canadian Pacific Railway and the Trans

Canada Highway) which are known to account for a high percentage of the mortality of wolverines in this area (J. Krebs, personal communication). The Revelstoke region also has more human inhabitation around it than the Williston Lake region. These and other anthropogenic factors may contribute to decreased gene flow to and from the more southerly population. Our results may also reflect that the Revelstoke population simply has a smaller effective population size than more northern populations.

Potential isolation of the Idaho population

The decreased level of H_E and the increased levels of genetic structure based on the assignment test, F_{ST}, D_S and D_{LR} in Idaho are consistent with other studies which have identified insular populations. In a study of black bears (*Ursus americanus*) Paetkau and Strobeck (1994) found H_E to be » 80% in all regions with one exception. Bears found in Terra Nova National Park, which are isolated on the island of Newfoundland, were found to have an H_E of 49%. A study of brown bears by Peatkau et al. (1998) found H_E to range from » 60 to 80% with the exception of two insular populations, Kodiak Island, AK and Yellowstone National Park which had H_E values of 26.5 and 55.4%, respectively. The decreased genetic variability of the Yellowstone brown bears was attributed to population fragmentation in the early 1900s from the larger continuous population in the north. Here, the lack of genetic variation in the Idaho population, relative to the homogenous levels of variation in most other regions sampled suggests that this population may have become fragmented from the larger distribution of wolverines in the north (Table 2-1). An alternative to this suggestion would be that a historical bottleneck is responsible for the lack of genetic variation found in this population. Although wolverine numbers are thought to have been declining in the southern reaches of their distribution since the 1840s (Hash 1987), there is no evidence that a historical population bottleneck has caused this decrease in genetic variation.

The results from the assignment test (Table 2-2) also support our suggestion that the Idaho population has restricted gene flow and may be fragmented from the other sampled regions. The Idaho population is only 700 km from the Revelstoke population, yet it reveals no crossassignments to Revelstoke, or any other regions. This would suggest that there is a potential barrier to gene flow between these two areas other than isolation by distance.

The genetic measures of F_{ST}, D_S and D_{LR} (Tables 2-3 and 2-4) all revealed that the Idaho population was the most genetically distinct region sampled. The genetic distance estimates from Idaho to all other regions are not proportional to the geographical proximity of this population to others (Fig. 2-3). When values of D_{LR} were entered into a three-way Mantel test, controlling for distance, it was found that these values were strongly and significantly associated with a barrier to gene flow ($r = 0.932$ $P = 0.0012$).

In the conterminous US wolverines are only thought to persist in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California from a once continuous population ranging as far south as Arizona and New Mexico (Hash 1987), supporting the suggestion that this population may be fragmented. The Idaho population also presents a similar example to that of insular Yellowstone brown bears (Paetkau et al. 1998). A recent study by Edelman and Copeland (1999) also suggests that the central Idaho population may be isolated from other north-west US wolverine populations. As wolverine dispersal is not thought to be limited by topographical features (Banci 1994), the fragmentation of southern populations may be attributed to habitat loss, overharvest and other anthropogenic factors as suggested by Banci (1994) and Wilson (1982).

In the American north-west, more populations will be needed to determine if these regions all represent genetically insular groups or if gene flow does occur among them and the more northern populations. It is not clear whether other remaining north-western US populations are presently self-sustaining populations or if they are dependent on emigration from Canadian populations (Montana, Newby and McDougal 1964; Idaho, Groves 1988; Oregon, Johnson 1977). It would appear that the surveyed Idaho population is not maintained by Canadian immigrants, as they are genetically distinct from all other sampled populations. It should be noted, however, that the most likely dispersal corridors for wolverines in the American northwest run north/south in the mountain ranges, as most valleys are developed with transportation corridors and human habitation, potentially restricting wolverine movements. The north/south corridor from Idaho to Canada would run into Glacier National Park in southern Alberta. Samples were not available from this region and so we were unable to determine whether animals from central Idaho are genetically connected to them.

The conclusions from the analyses of the Idaho populations must be tempered, however, due to the fact that only 14 individuals were sampled. A small sample size may potentially skew results by providing genotype frequencies that do not reflect those actually present in the population and making the population appear more distinct than it is. A further bias may also have been introduced into our results by including two known relationships in this sample. A potential father was also identified for these relationships. Despite the inclusion of these relationships we still believe that the Idaho population is genetically depauperate as all other potential fathers for the identified mother/offspring relationships matched at 13–15 of 16 loci (data not shown), reflecting the level of genetic relatedness in this region. Furthermore, running the data with and without the known relationships had little effect our presented results (data not shown).

Eastern populations

In the east only one population from Manitoba was sampled. This population was found to be genetically distinct from all other regions in this study. The two-way Mantel tests suggest that the genetic distances observed for this population are consistent with isolation by distance. However, with no immediately adjacent populations sampled in the east we are unable to say that Manitoba is not genetically isolated from other wolverine populations.

Conclusions

Although this study elucidated the levels of genetic structure between wolverine populations, we are unable to fully describe the likely complicated local social structure of wolverines. Local wolverine groups, which may exist with juvenile females establishing home ranges adjacent to their mothers, will not be revealed by sampling over a broad scale, as in this study. This study has shown that populations of wolverines that are exposed to fewer anthropogenic factors potentially have much gene flow among them. In direct contrast, the southern populations of Idaho and to a certain degree Revelstoke, which are exposed to greater pressure from humans (settlement, road systems, recreation in remote areas, and so on), seem to be more genetically isolated. The viability of these populations seems to be dependent, to a great extent, on large areas of undisturbed habitat and corridors among them.

Acknowledgements

I would like to thank the following people for providing samples for this study: John Krebs and Davis Lewis from the Columbia Basin Fish and Wildlife Compensation Program, Don Reid and Eric Lofroth (British Columbia Ministry of the Environment) from the Northern Wolverine Project, Rob Mulders and Albert Bourque from the Department of Resources, Wildlife and Economic Development, Northwest Territories, the University of Alaska Museum (Dr J. Cook and Dr G. Jarrell), Jeff Copeland, Idaho Fish and Game, Neil Dawson (Ontario Ministry of Natural Resources), and Barry Verbiwski (Manitoba Wildlife Branch).

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Table 2-1. Genetic variation estimates of wolverines using twelve microsatellites from sampled regions including the mean number of alleles (A), the mean unbiased expected heterozygosity (H_e), and the unbiased probability of identity (P_{ID}) using twelve microsatellites.

	Abbreviation	N	A	H_e	P_{ID}
Anchorage,AK	Anc	36	5.17	65.45%	6,450,000,000
Arctic, AK	Arc	47	5.83	66.90%	10,190,000,000
Nabesna, AK	Nab	38	5.25	68.48%	36,100,000,000
Russian Mission, AK	Rus	34	5.00	63.53%	1,647,000,000
Noatak, AK	Noa	38	5.00	65.31%	5,090,000,000
Nome, AK	Nom	35	4.67	62.31%	1,004,000,000
Bay Chimo, NU	Bay	40	4.83	63.61%	1,278,000,000
Kugluktuk, NU	Kug	67	5.00	64.68%	2,540,000,000
Revelstoke, BC	Rev	47	5.17	60.98%	377,000,000
Williston Lake, BC	Wil	37	4.92	61.18%	1,231,000,000
Idaho	Ida	14	2.83	42.09%	452,000
Manitoba	Man	28	4.50	66.99%	1,865,000,000

Table 2-2. Population assignments from the genotype assignment test. Far left column are regions sampled from, top row are populations individuals assigned to.

	N	Anc	Arct	Nab	Rus	Noa	Nom	Bay	Kug	Rev	Will	Ida	Man
Anc	36	8	7**	1	7	2	2	0	5*	1	1	0	1
Arc	47	4	10	4	4	9*	3	6*	1	1	4	0	1
Nab	38	5	4	11	2	9**	2	1	3	0	1	0	0
Rus	34	3	7**	3	6	4	6*	2	2	1	0	0	0
Noa	38	2	5	3	7*	13	2	1	2	1	2	0	0
Nom	35	2	3	2	2	1	21	2	2	0	0	0	0
Bay	40	0	2	1	7**	2	0	19	7	0	0	0	2
Kug	67	2	1	2	3	3	3	20*	23	0	5	0	5*
Rev	47	1	2*	0	1	0	0	2	1	35	4	0	1
Wil	37	3	0	2	3	3	3	1	1	5	15	1*	0
Ida	14	0	0	0	0	0	0	0	0	0	0	14	0
Man	28	0	0	0	0	1	1	0	3*	0	0	0	23

[*] significant at the 5% level for randomizations within each gene pool.

[**] significant at the 1% level for randomizations within each gene pool

Table 2-3. Upper diagonal represents pairwise F_{st} values (Weir and Cockerham 1984); lower diagonal as calculated by the Genepop 3.1d software package. Lower diagonal is the approximate geographic distances (km) between populations as calculated by the program DistGeo using approximate latitudes and longitudes of from the center of each sampled region.

	Anc	Arc	Nab	Rus	Noa	Nom	Bay	Kug	Rev	Wil	Ida	Man
Anc		.000	.010	.002	.009	.018	.035	.020	.072	.034	.209	.090
Arct	730		.012	.007	.004	.025	.026	.018	.061	.030	.196	.082
Nab	390	560		.013	.009	.023	.043	.029	.068	.030	.179	.082
Rus	590	980	940		.009	.017	.027	.014	.063	.028	.216	.092
Noa	950	865	1140	575		.025	.030	.017	.065	.021	.195	.094
Nom	875	970	1135	390	230		.041	.024	.069	.041	.208	.102
Bay	2235	1665	1870	2640	2470	2615		.007	.060	.047	.207	.092
Kug	1760	1205	1395	2170	2035	2170	480		.053	.027	.192	.096
Rev	2245	2285	1940	2840	3070	3075	1920	1780		.036	.169	.121
Wil	1605	1670	1295	2200	2430	2430	1700	1415	645		.167	.111
Ida	2885	2980	2610	3475	3745	3735	2515	2445	700	1320		.184
Man	3210	2860	2820	3735	3720	3820	1435	1730	1595	1900	1800	

Table 2-4. Genetic Distances: likelihood ratio genetic distance (D_{LR}, Peatkau *et al.* 1997) in the lower diagonal and Nei's standard genetic distance (D_S, Nei 1972) in the upper diagonal.

	Anc	Arc	Nab	Rus	Noa	Nom	Bay	Kug	Rev	Wil	Ida	Man
Anc		0.04	0.05	0.03	0.04	0.06	0.09	0.06	0.16	0.08	0.36	0.15
Arc	0.42		0.04	0.04	0.03	0.07	0.07	0.05	0.14	0.08	0.32	0.12
Nab	0.43	0.47		0.05	0.04	0.06	0.10	0.07	0.14	0.06	0.27	0.14
Rus	0.25	0.24	0.55		0.04	0.06	0.07	0.05	0.14	0.07	0.35	0.13
Noat		0.47	0.44	0.61		0.07	0.08	0.05	0.14	0.06	0.30	0.15
Nom	0.8	1.11	0.99	0.7	1.18		0.10	0.07	0.15	0.09	0.33	0.16
Bay	1.39	0.92	1.63	0.93	1.22	1.76		0.03	0.13	0.11	0.33	0.12
Kug	0.83	0.85	1.05	0.65	0.94	1.2	0.25		0.12	0.07	0.31	0.13
Rev	3.2	2.83	2.98	2.6	2.99	3.3	2.59	2.14		0.08	0.21	0.18
Wil	1.12	1.22	1.08	1.09	1.14	1.71	1.59	1.02	1.35		0.20	0.15
Ida	8.53	8.04	7.01	8.44	7.51	8.87	8.42	7.78	5.71	5.05		0.35
Man	2.33	2.22	2.75	2.34	2.9	2.98	1.98	1.92	3.15	2.59	8.14	

Figure 2-1. Map of Sampled Regions

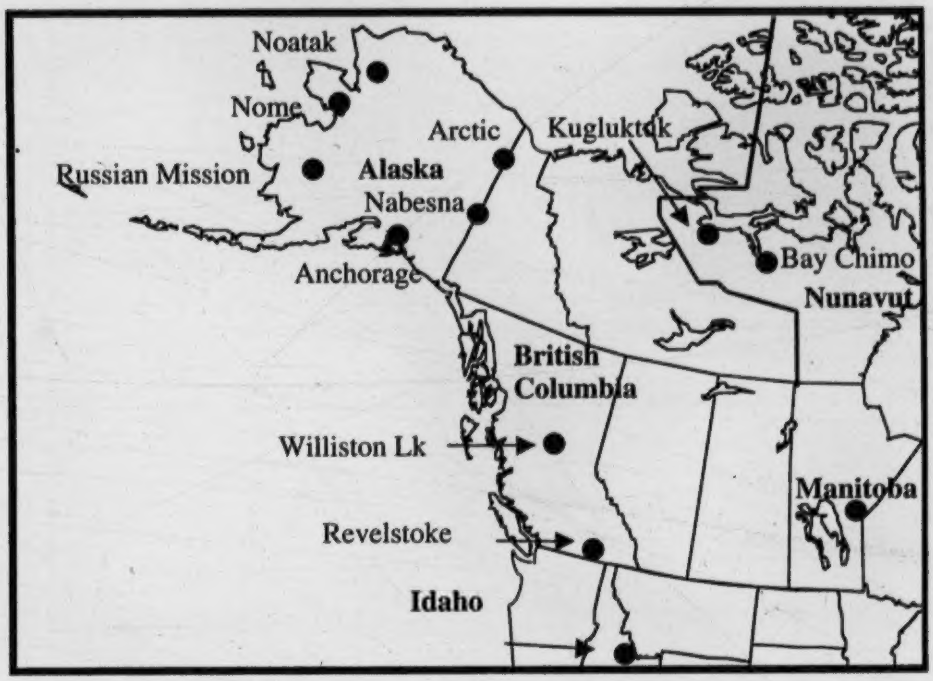


Figure 2-2. Unrooted neighbour-joining tree of genetic distances, D_S (Nei's standard). The length of the tree branches are relative to the genetic distances.

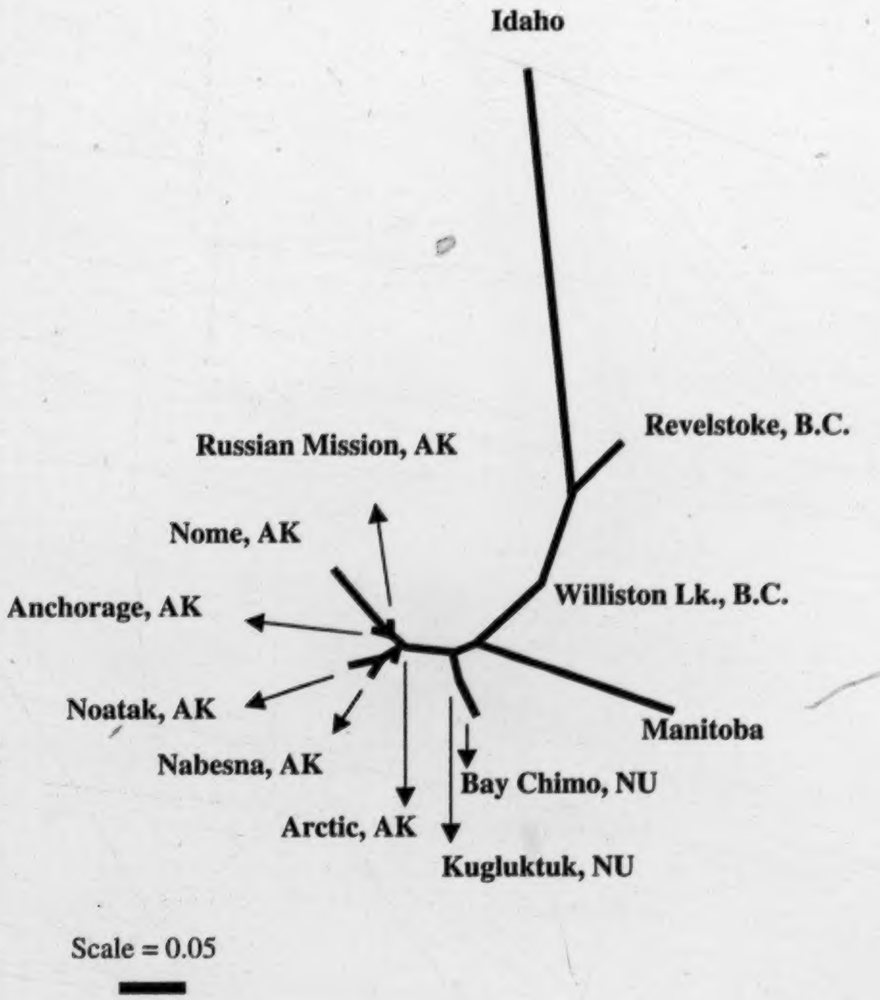
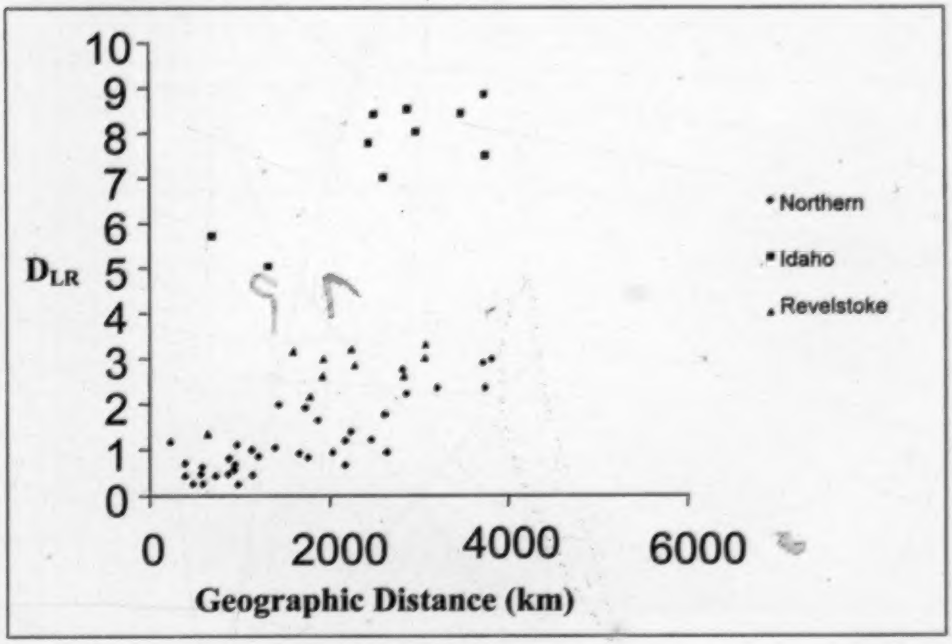


Figure 2-3. Relationship of likelihood ratio genetic distances, D_{LR}, to geographical distances.



Chapter 3

Connectivity of Peripheral and Core Populations of North American Wolverines*

Introduction

Wolverines are an enigmatic and rarely observed mustelid, inhabiting the tundra, taiga, and forest zones of portions of North America and Eurasia (Wilson 1982). In North America, the abundance and distribution of the species has declined since the advent of European settlement, most notably in eastern Canada, Vancouver Island, southern Rocky Mountains, and California (Banci 1994; Wilson 1982). Historical persecution (trapping and poisoning) and displacement from native habitat may be responsible for these declines. As a result, wolverines have been granted special conservation status across much of their current range. In Canada, the eastern population is listed as endangered (although potentially extirpated); whereas, the western population is granted special concern status (COSEWIC 2001). In the contiguous United States, petitions have been put forth to list the wolverine as endangered; to date these petitions have been unsuccessful due to the lack of adequate information supporting this designation (http://www.wolverinefoundation.org).

Wolverines are highly vagile creatures, with males and females able to disperse vast distances in a relatively short period of time (Copeland 1996; Gardner 1985; Magoun 1985; Vangen et al. 2001). This characteristic has prompted the theory that wolverines may exist as a single panmictic unit in North America. In northern North America, this is true (Kyle and Strobeck 2001) as high levels of gene flow were observed among populations from western Alaska to central Nunavut. In contrast, relatively high levels of genetic structure were found between southern and northern populations (Kyle and Strobeck 2001). It was proposed that increased anthropogenic pressures (e.g., fur harvest, habitat destruction, heavily traveled transportation corridors) on the southern populations could be reducing the level of gene flow between some populations of this species.

A study of Scandinavian wolverines from northern and southern Norway and Sweden by Walker et al. (2001) reported findings similar to those found for southern North American populations (Kyle and Strobeck 2001). Using microsatellites, Walker et al. (2001) found significant levels of genetic structuring between wolverine populations ($F_{ST} = 0.045$) and low estimates of genetic variation ($H_E = 39\%$). They attributed their results to long-term anthropogenic influences such as predator removal programs and hunting, that reduced the number and range of wolverines in Scandinavia prior to 1970 (Landa and Skogland 1995). However, subsequent protective legislation resulted in increasing wolverine densities in these regions (Landa 1997), and

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***A version of this chapter is in press. Kyle, C.J., and C. Strobeck. Journal of Mammalogy.**

the recent re-colonization of southern Norway. The genetic distinctiveness of this population was ascribed to a founder effect.

This study expands upon previous work by investigating the genetic structure of additional northern wolverine populations, populations on the edges of their current North American distribution, and 1 population in eastern Russia. Our goal was to determine if populations on the periphery of the current distribution of wolverines, where their historic ranges and numbers have been reduced in the last century, are genetically isolated from the larger continuous core of populations. In such regions, the influences of genetic drift could lead to genetically distinct populations, especially for this mid-sized carnivore existing at a low population density (Banci 1994). If these peripheral populations are found to be small fragments of a larger, continuous core of northern wolverine populations, they may be more susceptible to stochastic events leading to local extirpation (Hanski 1999). As such, these regions would be appropriate for concerted conservation efforts to re-establish connectivity between fragmented populations and the larger continuous distribution of animals.

Materials and Methods

Sample collection

This study combines the data of Kyle and Strobeck (2001) from: Alaska, Nunavut, Williston Lake (British Columbia), Revelstoke (British Columbia), Manitoba, and Idaho, with samples from 10 additional geographic regions. Hair, pelt, and tissue samples were obtained from eastern Russia, the Whitehorse region in the Yukon, the Fort Rae and Rennie Lake regions of the Northwest Territories, the Smithers and Prince George regions of British Columbia, the Grande Cache region of Alberta, northwestern Saskatchewan, northwestern Ontario, and the Yellowstone region of Wyoming (Fig. 3-1).

Laboratory methods

DNA was extracted using a DNAeasy® Tissue Extraction Kit (QIAGEN). Twelve polymorphic microsatellites were amplified using primers developed in badgers (BA-1, BA-4; Davis and Strobeck 1998), martens (MA-3; Davis and Strobeck 1998), wolverines (GG-3, GG-4, GG-7, GG-14; Davis and Strobeck 1998; and Ggu 101, Ggu 216, Ggu 234; Duffy et al. 1998), mink (Mvis-75; Flemming et al. 1999), and Eurasian otters (L-604; Dallas and Piertney 1998). Amplification conditions are given in Davis and Strobeck (1998). DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer (PE Applied Biosystems Inc., Foster City, California). DNA fragments were analyzed using the programs GeneScan™ Analysis 2.02 and Genotyper® 2.0 (PE Applied Biosystems Inc., Foster City, California).

Data analysis

A *G*-test for heterogeneity of allele distributions, averaged across all loci (Sokal and Rohlf 1995), was performed for each pair of the sampled areas. Departures from Hardy-Weinberg equilibrium (HWE) were tested for each of the 12 loci using Genepop 3.1d (Raymond and Rousset 1995) that uses a Markov chain method following the algorithm of Guo and Thompson (1992). Genepop also was used to evaluate genotypic disequilibria among loci.

Relative genetic variation in each population was assessed using mean number of alleles and unbiased expected heterozygosity, H_E (Nei and Roychoudhury 1974). Pairwise genetic distances were estimated using Nei's standard genetic distance, D_S (Nei 1972), calculated from genotype frequencies, and the genotype likelihood ratio distance, D_{LR} (Paetkau et al. 1997), calculated from genotype probabilities. Both pairwise distances were calculated by programs designed by John Brzustowski (http://www.biology.ualberta.ca/jbrzusto/Doh/php). Pairwise F_{ST} estimates were obtained from the software package Genepop 3.1d (Raymond and Rousset 1995, as per Weir and Cockerham 1984).

An unrooted neighbor-joining tree of D_S values was created using PHYLIP 3.573 (Felsenstein 1995). The programs Seqboot (1000 bootstraps) and Consense (PHYLIP) were used to obtain statistical support for the neighbor-joining tree. The association between geographic and genetic distance values were tested with a two-way Mantel test (Mantel 1967; http://www.fas.umontreal.ca/BIOL/legendre/) and regressions of the data were performed to obtain comparisons of genetic structure per unit geographic distance.

The assignment test (Paetkau et al. 1995; http://www.biology.ualberta.ca/jbrzusto/Doh/php) was conducted on all populations. This program determines the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in each of the other regions included in the test. Individuals are then assigned to the population where their genotype has the highest probability of occurring (see Waser and Strobeck 1998). Randomizations (10, 000) of the data were performed within each gene pool assuming HWE. This test identifies cross-assignments that are unlikely to have occurred due to chance alone, thus identifying individuals that may be migrants.

Results

Tests of disequilibria

After accounting for sample-wise error (Dunn-Sidak method; Sokal and Rohlf 1995), 1 departure from HWE was found at locus L-604 in the Yukon population. Heterozygote deficiencies were responsible for this result, suggesting the presence of null alleles in this population. Because L-604 conformed to HWE in all other populations, it was retained for further analyses.

Genotypic disequilibrium was observed for 2 pairs of loci in the Alberta population: GG3 with GG4, and BA1 with L604. As genotypic disequilibria were not found for these loci in any other population, it is unlikely that they are physically linked and were therefore retained for further analyses. This result may reflect the presence of a number of related individuals in the Alberta population.

Heterogeneity of sampled regions

Due to the broad sampling scheme, large number of regions sampled, low pairwise genetic distances and F_{ST} values, and for ease of comparison, all adjacent regions that did not differ significantly in their genotypic frequencies ($\alpha = 0.005$) were pooled (c.f. Kyle and Strobeck 2001 where $\alpha = 0.05$). Hence, the Williston Lake, Prince George, and Smithers regions were combined into 1 population and referred to as central British Columbia; the Rennie Lake and Fort Rae regions of the Northwest Territories were combined into 1 population; all Alaskan regions were pooled, and the Kugluktuk and Bay Chimo regions of Nunavut were pooled. Additional details on the genetic variation within and among the Alaskan and Nunavut populations can be found in Kyle and Strobeck (2001).

Genetic variation

The mean number of alleles ranged from 2.8 (Idaho) to 5.7 (Russia). The average level of heterozygosity (H_E) among all sampled regions was 61% (Table 3-1). At 42%, Idaho was the only population with a significantly lower level of genetic variation (pairwise comparisons; Wilcoxon's signed-ranks test, Sokal and Rohlf 1995).

Pairwise F_{st} and genetic distance measures

Pairwise F_{st} values ranged from essentially zero to 0.235 (Table 3-2). Among the northern regions (Alaska, Yukon, Northwest Territories, Nunavut, central British Columbia, and northern Saskatchewan) pairwise F_{ST} values ranged from essentially zero to 0.029. F_{ST} among eastern (Manitoba and Ontario) populations was relatively low (0.012), and moderate between eastern and northern populations (0.032 -- 0.057). Among the southern regions (Revelstoke, Idaho, and Wyoming) the pairwise values were relatively high (0.047 -- 0.147). These values were even higher when comparing southern to northern regions (0.034 -- 0.200), with the exception of comparisons between southern and Alaskan populations where the F_{ST} values were relatively low (0.004-0.015). The lowest values observed between Russian and North American populations were to Alaska (0.038). All other comparisons between Russia and North America yielded relatively high values (0.116 -- 0.235).

The genetic distances, D_{LR} (data not shown) and D_S (Table 3-2), were found to correlate to one another by a two-way Mantel test ($r = 0.72$, $P = 0.0004$). D_S values ranged from about 0.021 to 0.490. The D_S values paralleled and were correlated to the findings from the F_{ST} estimates ($r = 0.77$, $P = 0.0005$). The main exceptions were the pairwise D_S values between Russia and North America; unlike the F_{ST} values, D_S was relatively consistent, ranging from 0.287 -- 0.490. An unrooted neighbor-joining tree, based on pairwise D_S values (Fig. 3-2) summarizes relationships between populations.

In this tree the Idaho and Russian populations appear nearly equally distinct from the northern North American populations.

Pairwise geographic and genetic distance (D_S) were plotted against each other (Fig 3-3). Using a two-way Mantel test, geographic and genetic distance, D_S, were correlated to each other ($r = 0.52$, $P < 0.0001$). Fig. 3-4 illustrates the relative genetic structure of several carnivores to that of wolverines, in relation to geographic distance. The steepest slopes were found in brown bears (0.137/1000km, SE 0.013; Paetkau et al. 1998) and fishers (0.092/1000km, SE 0.008; Kyle et al. 2001), whereas the lowest values were obtained for wolverines (0.0183/1000km, SE 0.005), and northern martens (0.057/1000km, SE 0.009; Kyle et al. 2000).

Assignment test

Of the Russian samples processed, 97% of the individuals were assigned to Russia (Table 3-3). For the 2 cross-assignments observed from Russia to North America, assignment probabilities suggest that the individuals were nearly as likely to occur in Russia as North America (Fig. 3-5a).

In the northern regions, the vast majority of cross-assignments went to other northern regions. In eastern populations, most cross-assignments were exchanged between the 2 populations in Ontario and Manitoba, with the remainder being assigned to northern regions.

The southern populations, compared to northern regions, produced fewer cross-assignments with the exception of Wyoming. In this population, 50% of the assignments were assigned to northern populations (Figs. 3-5b and c).

Discussion

Wolverine populations in northern North America experience high levels of gene flow. This is consistent with the impressive dispersal abilities of both male and female wolverines (Copeland 1996; Gardner 1985; Magoun 1985; Vangen et al. 2001), and with a previous study by Kyle and Strobeck (2001). However, genetic structure in the southern and eastern extremes of the distribution of wolverines is relatively high, possibly reflecting the fragmented nature of these populations at the periphery of their historical range.

Russian population

The Russian wolverine population was found distinct from all North American populations (Tables 3-2 and 3-3) with the few cross-assignments observed from Russia to North America likely occurring due to chance (Fig. 3-5a). However, rare transcontinental migration events may not be impossible for this species. Arctic foxes are known to cross the Bering Sea via St. Lawrence Island on the pack ice (Fay and Stephenson 1987) so it may also be possible for wolverines. In general, however, Russia and Alaska have been considered isolated for >10, 000 years, and observed genetic distance/F_{ST} results may provide a baseline for evaluating isolation among North American populations.

Northern regions

Little genetic structure exists among northern regions (Tables 3-2 and 3-3), suggesting the occurrence of extensive gene flow. These findings are expected given the life history characteristics of wolverines, including their capacity to move long distances with relative ease as documented by Copeland (1996), Gardner (1985), Gardner et al. (1986), and Magoun (1985) and the presence of relatively continuous habitat. It appears that these characteristics have had the effect of genetically homogenizing wolverine populations in habitats with relatively few anthropogenic influences.

An alternative to the aforementioned hypothesis is the lack of structure in northern regions may reflect a relatively recent post-glacial colonization of this area. However, wolverines were thought to exist in several refugia during the Wisconsin glaciation, both in regions south of the ice sheets and in Beringia (fossil evidence; Bryant 1987). Genetic trends currently observed have most likely not been influenced by a rapid radiation of wolverines northward from a southern glacial refugium, but reflect recent and consistent migration events.

Our results conflict slightly with a previous study by Wilson et al. (2000) who investigated the genetic structure of wolverines from the Northwest Territories and found a moderate level of genetic structure using enzymes ($F_{ST} = 0.076$) and relatively strong structure with mtDNA ($\Phi_{ST} = 0.536$.).

Western Canadian populations

A high level of gene flow was found to occur between most western Canadian populations and regions further north suggesting that they are part of the nearly continuous northern distribution of wolverines. However, as suggested by Kyle and Strobeck (2001), the Revelstoke population in British Columbia shows signs of genetic isolation from more northern populations. The apparent lack of gene flow to and from Revelstoke may reflect heightened anthropogenic pressures in this region. Studies by Alexander and Waters (2000) and Austin (1998) indicate that the Trans Canada Highway (running through Revelstoke) acts as an impediment to movement for wolverines (among other species). Furthermore, historical effects from predator control programs (Hancock 1987) and trapping the early to mid 1900's, have reduced, and thus to some extent, isolated populations in this region. This and the effects of changes as a result of transportation development may, in part, be responsible for the current levels of genetic structure observed.

Eastern populations

All results suggest that the 2 eastern populations, Manitoba and Ontario, are quite similar to each other, but relatively distinct from the other regions. Due to the their proximity to the prairies (vast expanse of unsuitable habitat), Hudson's Bay, and the fact that these populations are on the periphery of the current distribution of wolverines, gene flow between these eastern populations and northern sources may be limited. This is reinforced partially by the genetic distances and number of cross-assignments to northern regions.

Southern populations

The Idaho population appears genetically distinct from all other regions. Interestingly, the Idaho and Russian populations are nearly as genetically distant from northern regions (Fig. 3-2). However, the genetic distances to the northern North American populations from Russia are the result of isolation since the last glaciation. In contrast, the genetic distances between Idaho and other North American populations may represent more recent (early to mid 1900's) population fragmentation or founder events. Historical persecution and displacement from native habitat were thought to have led to the extirpation of wolverines from much of the lower 48 states by the 1920's, including Idaho (Davis 1939), Montana (Newby and Wright 1955; Newby and McDougal 1963), and Washington (Johnson 1977). Thus, current populations in Montana and Idaho are a result of a relatively recent expansion from the north, or an expansion of small populations that may have been reduced to minimum levels during the early to mid 1900's. In either case, the likely small effective size of the Idaho population has led to rapid genetic drift and the current distinctiveness of this population.

The 1 cross-assignment from the Idaho population went to Nunavut (Table 3-3). While we are not suggesting that an individual migrated from Nunavut to Idaho, the individual genotype was much more likely ($\alpha<0.05$) to be from a more northern population than Idaho (probability of 10^{-19} in Idaho vs. $10^{-12} - 10^{-14}$ for northern populations, individual probability data not shown, see Fig. 3-5b). Given the potential for dispersal in this species, it is not unreasonable to suggest that there is some amount of gene flow between central Alberta or British Columbia and Idaho. Although less likely, it should be mentioned that re-introductions to the lower 48 states have taken place with wolverines originally obtained from northern Canada (e.g. John Denver and Stouffer Productions of Aspen, Colorado released 2 Canadian wolverines into the Maroon Bells-Snowmass Wilderness area of Colorado in 1978, Murray 1987).

The results for Wyoming were mixed with several individual genotypes resembling northern genotypes, while others appeared distinct from all other regions sampled. The cross-assignments from Wyoming to the northern regions were highly significant ($\alpha<0.05$; Fig. 3-5c). The lack of samples from intermediate populations in Montana makes it unclear if there is some amount of gene flow between Wyoming and Canadian populations.

Trends in other carnivores

The genetic structure observed in wolverines is similar to that of other carnivores; American pine martens, *(Martes americana)* sampled from the Canadian north show little population genetic structure between populations (Kyle et al. 2000). A similar result was also obtained in lynx (*Lynx* *canadensis*) sampled from across their North American distribution. In lynx, like wolverines, a high potential for dispersal is thought to be responsible for the lack of genetic structure across vast distances (Schwartz et al. 2002). Although more structure exists between brown bear (*Ursus arctos*) populations than between wolverine populations, Paetkau et al. (1998) found that populations in the southern reaches of the bears range were more genetically distinct, and showed less genetic variation than bears occupying less disturbed northern habitats. The parallels between the genetic structure of wolverines and brown bears at the southern periphery of their range may be correlated with low densities of humans and roads (Carroll et al. 2001).

Conclusions

Our results confirm that high levels of gene flow do occur among northern wolverine populations. We also observe progressively increasing genetic structure at the southern and eastern peripheries of their current distribution. Historical persecution and displacement from native habitat may have resulted in smaller populations, partially fragmented from what was once, likely, a panmictic unit. Small, isolated populations are more susceptible to extirpation (Hanski 1999) and, therefore, should be targets of concerted conservation efforts. To reverse these trends, consideration should be given to re-establishing gene flow between peripheral and core populations to ensure the persistence of this species in regions under increasing anthropogenic pressure.

Acknowledgements

I would like to thank the following people and institutions for providing samples for this study: J. Krebs and D. Lewis (Columbia Basin Fish and Wildlife Compensation Program), D. Reid and E. Lofroth (British Columbia Ministry of the Environment; Northern Wolverine Project), R. Mulders and A. Bourque (Department of Resources, Wildlife and Economic Development, NWT), J. Cook and G. Jarrell (University of Alaska Museum), J. Copeland (Rocky Mountain Research Station, Missoula, Montana), K. Pilgrim, F. Mallory, N. Dawson (Ontario Ministry of Natural Resources), B. Verbiwski and D. Berezanski (Manitoba Wildlife Branch), F. Corbould (Peace/Williston Fish and Wildlife Compensation Program), Alberta Trapper's Association, R. McClymont and B. Trichel (Alberta Fish and Wildlife), Dale Pederson, G. Mowat (Aurora Wildlife Research), and W. and M. Sharpe (North American Fur Auctions).

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Table 3-1. Genetic variation of wolverines as revealed by microsatellite data (N= sample size, A=mean number of alleles, and H_E=average expected heterozygosity).

Population	**N**	**A**	**H_E**
Russia	64	5.7	63%
Alaska	228	5.2	65%
Yukon	23	4.7	65%
Northwest Territories	40	4.7	65%
Nunavut	107	4.9	64%
Central British Columbia	68	5.0	61%
Revelstoke	47	5.2	61%
Alberta	17	5.0	61%
Saskatchewan	15	4.3	64%
Idaho	14	2.8	42%
Wyoming	8	3.6	56%
Manitoba	28	4.5	67%
Ontario	12	3.8	63%
Total/Average	671	4.6	61%

Table 3-2.- Pairwise genetic distances for wolverine populations using microsatellite data: Nei's Standard Genetic Distance, D_S (lower diagonal) and F_{ST} (upper diagonal), see Fig. 2 for abbreviations.

	RU	**AK**	**YK**	**NT**	**NU**	**BC**	**RV**	**AB**	**SK**	**ID**	**WY**	**MB**	**ON**
RU	0	.038	.116	.127	.128	.138	.170	.126	.124	.235	.165	.145	.156
AK	.287	0	.003	.004	.007	.007	.015	.005	.001	.016	.004	.008	.003
YK	.298	.068	0	.018	.028	.028	.055	.026	.019	.159	.063	.032	.034
NT	.323	.053	.068	0	.002	.020	.035	0.16	.000	.176	.047	.037	.045
NU	.317	.041	.084	.021	0	.029	.054	.036	.000	.169	.060	.046	.057
BC	.323	0.44	.076	.053	.065	0	.034	.034	.026	.127	.049	.057	.055
RV	.433	.125	.131	.081	.116	.071	0	.051	.055	.141	.047	.078	.076
AB	.313	.124	.097	.065	.101	.087	.117	0	.026	.201	.057	.041	.043
SK	.327	.059	.086	.034	.034	.081	.135	.103	0	.200	.076	.031	.045
ID	.490	.290	.257	.303	.297	.188	.208	.311	.322	0	.147	.185	.234
WY	.433	.181	.181	.138	.165	.139	.126	.168	.218	.210	0	.094	.102
MB	.405	.119	.109	.101	.116	.127	.181	.129	.273	.330	.273	0	.012
ON	.437	.132	.126	.138	.158	.144	.185	.146	.149	.395	.264	.084	0

Table 3-3.- Genotype assignment test for wolverine populations. Left column represents where wolverines were sampled from, and top row represents where the animals were assigned to. Note that rounding of percentages may result in values not summing to 100%. Underlined cross-assignments were found to be significant by randomizations of individual gene pools assuming H.W.E. See Fig. 2 for abbreviations.

					% of individuals assigned								
	RU	**AK**	**YK**	**NT**	**NU**	**BC**	**RV**	**AB**	**SK**	**ID**	**WY**	**MB**	**ON**
RU	**96.9**		1.56					1.56					
AK	0.4	**56.1**	6.1	3.1	10.1	9.6	1.8	1.3	7.5		1.3	1.3	1.3
YK		4.3	**43.5**	13.0		17.4		4.3				13.0	4.3
NT		10.0	2.5	**20.0**	20.0	2.5	5.0	10.0	20.0			10.0	
NU		6.5	2.8	15.9	**49.5**	3.7	0.9	3.7	10.3			4.7	1.9
BC	1.5	8.8	5.9	8.8	4.4	**42.6**	10.3	4.4	2.9	2.9	2.9	2.9	1.5
RV		2.1	6.4	6.4		12.8	**61.7**		4.3	2.1	2.1		2.1
AB		5.9	5.9	23.5				**41.2**	11.8		5.9	5.9	
SK		13.3	7.7	13.3	26.7				**26.7**			13.3	
ID					7.1					**92.9**			
WY				12.5	12.5				25.0		**50.0**		
MB		14.3	3.6	7.1	3.6			3.6				**75.0**	17.9
ON			25.0									16.7	**58.3**

Figure 3-1. Map depicting 22 sampled localities of wolverines. Squares represent sampled localities from Kyle and Strobeck (2001), circles represent localities sampled in this study.

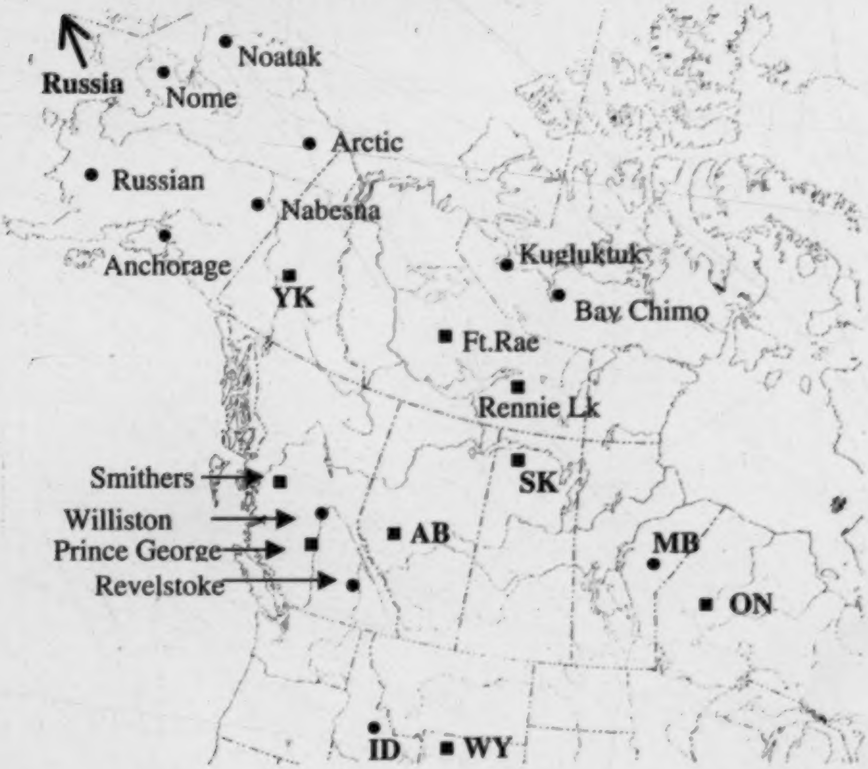


Figure 3-2. Unrooted neighbor-joining tree of Nei's standard genetic distance, D_S. The length of the tree branches are relative to the genetic distances. Only branch support (from 10, 000 bootstraps) of greater than 60% is shown. Localities include: Russia (RU); Idaho (ID); Wyoming (WY); Revelstoke (RV); central British Columbia (BC); Alberta (AB); Saskatchewan (SK); Manitoba (MB); Ontario (ON); Kugluktuk and Bay Chimo, Nunavut (NU); Fort Rae and Rennie Lake, Northwest Territories (NT); Whitehorse, Yukon (YK); Arctic, Anchorage, Nome, Nabesna, Noatak, and Russian Mission, Alaska (AK).

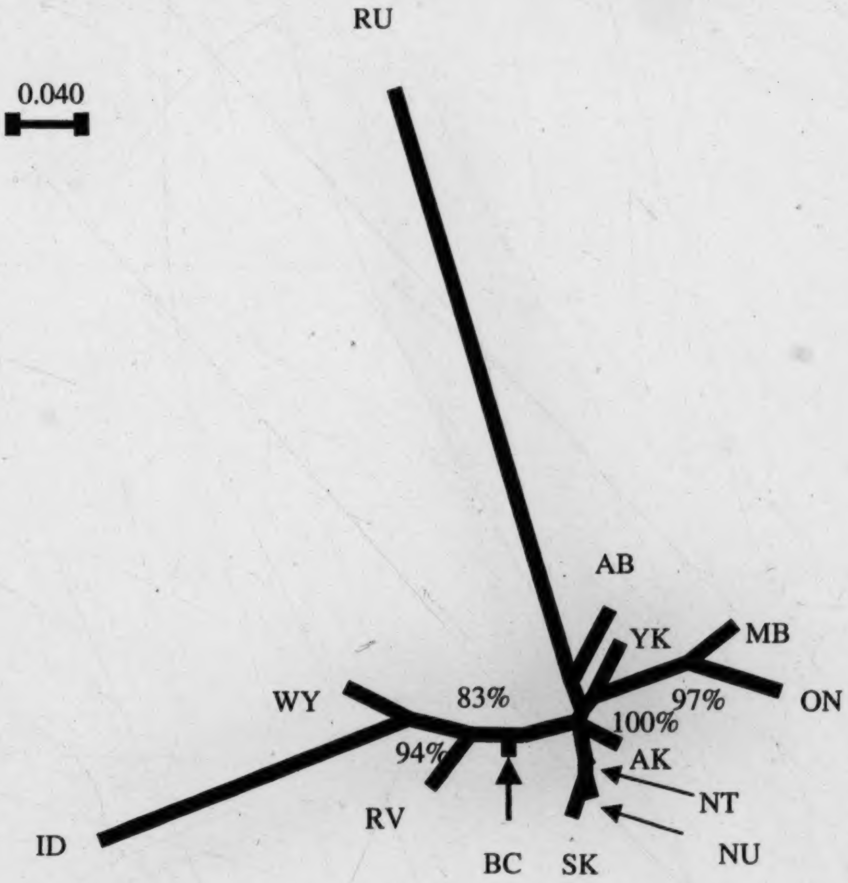


Figure 3-3. Plot of geographic distance (km) relative to Nei's standard genetic distance, D_S, for all sampled wolverine localities. Closed squares represent pairwise values to Russia, open triangles are pairwise values to Idaho, open diamonds are northern population pairwise values, and closed circles are pairwise comparisons to eastern populations (Ontario and Manitoba).

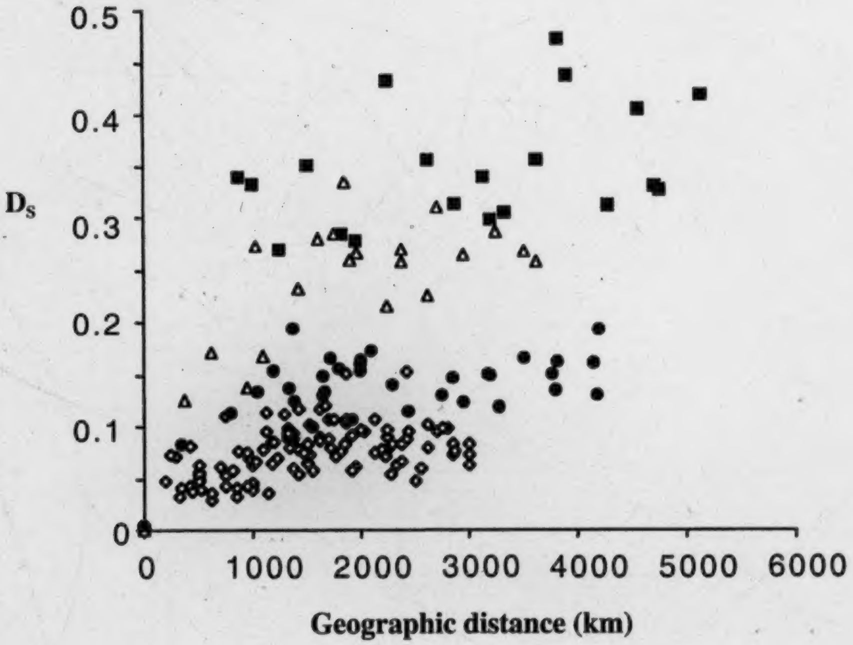


Figure 3-4. Plot of geographic distance (km) relative to Nei's standard genetic distance, D_S for wolverines (open circles), martens (closed triangles, Kyle et al. 2000), fishers (open squares, Kyle et al. 2001), and brown bears (stars, Paetkau et al. 1998). Peripheral populations and re-introduced populations with disproportionate genetic distances per unit geographic distance are excluded (brown bears, Wyoming and Kodiak Island bears excluded; fishers, re-introduced populations excluded; martens, all populations included; wolverines, Russia, Idaho, Wyoming, and Revelstoke populations excluded).

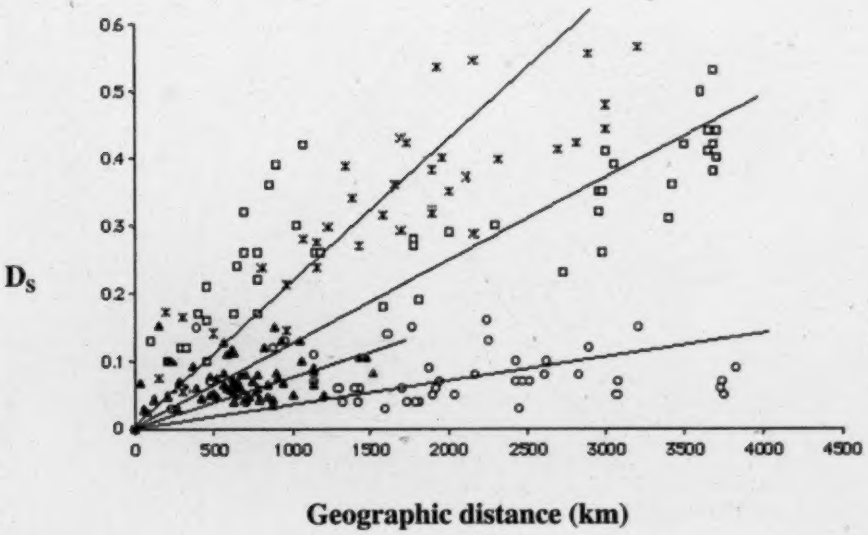
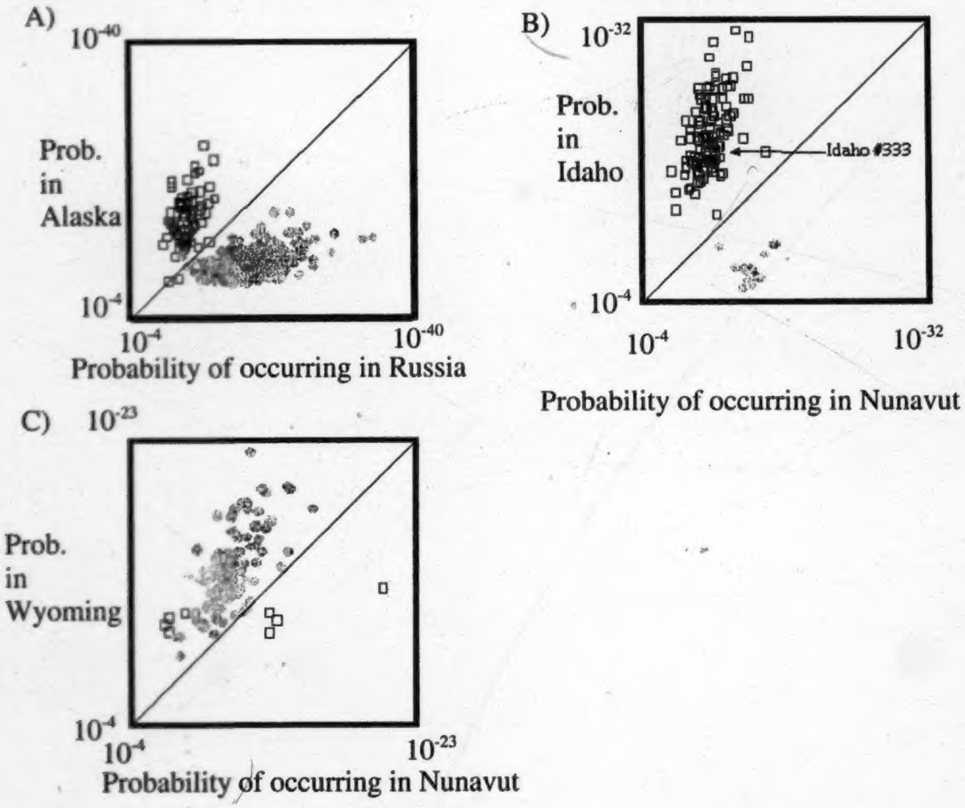


Figure 3-5. Pairwise plots of genotypic probabilities of occurring in either of the illustrated populations from genotype assignment test. Individuals found on the opposite side of the diagonal represent cross-assignments. a) Russia vs. Alaska, open squares are individuals captured in Russia, closed circles are individuals captured in Alaska. b) Idaho vs. Nunavut, c) Wyoming vs. Nunavut.



Chapter 4

Genetic variation and structure of fisher (*Martes pennanti*) populations across North America

Introduction

Fishers (*Martes pennanti*) are mid-sized forest carnivores indigenous to North America. Prior to European settlement this species was distributed throughout the forests of Canada and the northern United States, projecting southward along the Appalachian and Pacific Coast mountain ranges (Graham and Graham 1994). Fisher populations experienced sharp declines throughout much of their distribution between 1800 and 1940 (see review by Powell and Zielinski 1994). These declines were attributed to overharvesting and habitat destruction via logging and development (Brander and Brooks 1973, Powell 1993). Since this time, conservation efforts (closed trapping seasons, habitat recovery programs, and re-introductions) have allowed fishers to return to much of their former range (Powell 1993, Gibilisco 1994). Despite these efforts, however, fisher numbers are still considered low in the Rocky Mountains, the Pacific northwest, and in the central Appalachians (Aubry and Houston 1992, Gibilisco 1994).

The intentions of this study were three-fold: first we wanted to evaluate the levels of genetic variation found in re-introduced populations relative to the nearest adjacent indigenous populations. Second, to elucidate the population genetic structure of fishers across a potentially fragmented landscape. Thirdly, we wanted to compare the levels of population genetic structure in fisher populations relative to two closely related mustelid species, martens (*Martes americana*) and wolverines (*Gulo gulo*). The levels of genetic variation were expected to be lower in re-introduced populations relative to adjacent indigenous populations. With respect to the population genetic structure of the species, our initial supposition was that fishers would have an intermediate level of genetic subdivision among populations, relative to martens and wolverines, given their intermediate size, density, home-range size, and dispersal ability. However, many other life-history characteristics beyond the aforementioned have important influences on the population genetic structure of a species. Fishers are thought to prefer late-successional forests (Ruggiero et al. 1991), and avoid areas with little canopy cover (Thomas et al. 1993). The same has been suggested for martens (Buskirk and Ruggiero 1994), but wolverines seem less habitat specific (Banci 1994). The distribution of deep snow limits the distribution of fishers (Aubry and Houston 1992), but this is not true for either martens or wolverines.

Two previous studies have described genetic variation in fishers. Williams et al. (1999, 2000) studied northeastern United States fisher

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***A version of this chapter has been published. Kyle CJ, Robitaille JF, and C. Strobeck (2001) Molecular Ecology 10:2341-2347, 2001.**

populations using allozymes and found heterozygosity to range from 0.027 – 0.090. These values are similar to those found in other mustelid studies using allozyme markers (Hartl et al. 1988, Lidicker and McCollum 1997).

Although little genetic variation was revealed using allozymes, evidence was found to suggest that significant structure exists among the regions studied.

As previous studies have found little genetic variation, microsatellite loci were used in this study to investigate the levels of genetic variation and population genetic structure of fishers. Microsatellites have identified clear genetic differentiation in other mammalian studies where allozyme markers had little variation (e.g. *Ursus arctos*; Paetkau et al. 1998).

Materials and Methods

Sampled Locations

Pelt, tissue, and hair samples were collected from the following regions throughout much of the northern range of fishers (Figure 4-1): British Columbia (Peace, Omineca, and southern interior regions), Alberta (collected from the northern half of the province designated: north, central, east and west), Manitoba (southeastern), Ontario (Manitoulin Island and French River regions), Quebec (southeastern and Rimouski regions), the state of New York (Adirondack mountains), and Nova Scotia (southern and central regions). All samples are of recent origin.

Fishers are known to have been re-introduced to three of the sampled regions. On Manitoulin Island 17 fishers were introduced during the winter of 1980/81 from the Bancroft district of Ontario. This release may have been preceded by another release during 1979/80 (no associated documentation could be found). It is not clear if fishers were completely extirpated from the island prior to the re-introduction. Fishers were completely extirpated from Nova Scotia by 1922 (Bensen 1959, Dodds and Martell 1971). In central Nova Scotia several re-introductions occurred between 1947 and 1966 (total N=70) using animals originally from Maine (Dodds and Martell 1971; Doug Archibald, pers. comm.). In southern Nova Scotia 20 animals from ranch stock (original source unknown, Micheal Boudreau, pers. comm.) were introduced.

Extraction, Amplification, and Visualization of DNA

DNA was extracted from all samples using a QIAamp® Tissue Extraction Kit. (QIAGEN). The thirteen microsatellite primer sets were developed by: Davis and Strobeck (1998) in badgers (BA-1), martens (MA-1, MA-2, MA-19) and wolverines (GG-7, GG-14); by Duffy et al. (1998) in wolverines (Ggu 101, Ggu 216); by Flemming et al. (1999) in mink and ermine (Mvis-072, Mvis002, Mer095, Mer082); and by Dallas and Piertney (1998) in Eurasian otters (L-604). Note that the microsatellites found to be invariant in this species were excluded from this study and may bias measures of genetic variation relative to other species. PCR amplification was performed as in Davis and Strobeck (1998), and the DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. The programs GeneScan™ Analysis 2.02 and Genotyper® 2.0 were used to analyze the DNA fragments. Samples not amplifying more than two of thirteen loci were omitted from this study. Genetic analysis software used was able to accommodate missing data.

Tests of Disequilibrium and Heterogeneity

Departure from Hardy-Weinburg equilibrium (H.W.E.) and genotypic disequilibrium were assessed, for each of the thirteen loci, using Genepop 3.1 (Raymond and Rousset 1995). Multiple comparisons were accounted for using the Dunn-Sidak experimentwise error rate. A *G*-test for heterogeneity, summed among loci (Sokal and Rohlf 1997), was then performed for each pair of sampled areas.

Genetic Variation

The relative genetic variation in each population was assessed using allele frequency data; mean number of alleles, unbiased expected heterozygosity (H_E, Nei and Roychoudhury 1974), and unbiased overall probability of identity (P_{ID}, Paetkau et al. 1998) were calculated. Wilcoxon's signed-ranks test was used to test for significant differences in heterozygosity levels among populations (Sokal and Rohlf 1997).

Genetic Distances and pairwise F_{ST}

Genetic distances between populations was estimated using Nei's standard genetic distance, D_S, (Nei 1972) and the genotype likelihood ratio, D_{LR}, (Paetkau et al. 1997). Both D_S and D_{LR} were calculated using programs within the website, www.biology.ualberta.ca/jbrzusto/Doh.php, (designed by John Brzustowski). Genepop 3.1was used to calculate pairwise F_{ST} estimates (as per Weir and Cockerham 1984). A two-way Mantel test (Mantel 1967) (found on Pierre Legendre's webpage: http://www.fas.umontreal.ca/BIOL/legendre/) was used to evaluate the correlation between the genetic and geographic distances.

Assignment Test

The assignment test (Paetkau et al. 1995), also found on the aforementioned web site by John Brzustowski, was run for all populations. This test determines the probability of a genotype occurring in the region from which it was sampled, and the probability of it occurring in each of the other sampled regions. It then assigns each individual to the population in which that individual's genotype has the highest probability of occurring (see Waser and Strobeck 1998).

Results

Tests of Disequilibrium and Heterogeneity

All sampled regions conformed to H.W.E. (accounting for experimentwise error), with the exception of southern Nova Scotia at locus Mer095. This deviation from H.W.E. appears to be caused by an excess of homozygotes, possibly implying the presence of null alleles in this population at this locus, although other situations such as a Walhund effect could account for these findings.. Since all other regions conformed to H.W.E. at this locus it was retained for all analyses. Four deviations from genotypic equilibrium were discovered after accounting for experimentwise error: Ggu216/MA19 in the Manitoba region, MA2/Mer095 in the Manitoulin Island region, MA1/Mer082 in the southern Nova Scotia region, and MA1/Mvis002 in the western Alberta region. As no two pairs of loci were found to have genotypic disequilibrium in more than one population all loci were retained for all analyses.

G-tests indicate that the regions initially designated Alberta north, central, and west did not differ significantly ($\alpha=0.01$) in their genotypic frequencies. Therefore, the three regions were pooled into one population, called Alberta west. All other regions sampled differed significantly in their genotypic frequencies and were considered distinct populations for all subsequent analyses.

Genetic Variation

The observed levels of genetic variation are presented in Table 4-1. Although introduced populations show slightly lower levels of genetic variation relative to adjacent indigenous populations, Wilcoxon's signed-ranks test found only found two pairwise comparisons to be significantly different ($\alpha=0.05$): Manitoulin Island compared to French River, and central Nova Scotia compared to Rimouski.

Genetic Distances and Pairwise F_{ST}

D_S, D_{LR}, and the pairwise F_{ST} values (see Table 4-2 for D_S and F_{ST} values) were significantly correlated, with each other, and geographic distance (two-way Mantel test). The results are as follows: D_S and D_{LR}, $r=0.96$ ($p<0.001$), D_S and F_{ST}, $r=0.65$ ($p<0.001$). D_S with geographic distance, $r=0.75$ ($p<0.001$), and F_{ST} and geographic distance $r=0.50$ ($p<0.001$).

The slope of genetic (D_S) versus geographic distance was calculated for fishers, martens and wolverines using a linear regression of observed values (from this study, Kyle et al. 2000, and Kyle and Strobeck 2001). The slope for fishers was 0.092/1000km (S.E. 0.008). In martens and wolverines, the slope was 0.057/1000km (S.E. 0.009) and 0.0183/1000km (S.E. 0.0048), respectively.

Assignment test

Most individuals (70-96%) were assigned to the population from which they were sampled (data not shown). The vast majority of cross-assignments were to the nearest adjacent populations.

Discussion

Fishers, in contrast to other closely related mustelid species and other carnivores (martens, Kyle et al. 2000; wolverines, Kyle and Strobeck 2001; and brown bears, Paetkau et al. 1998), demonstrate relatively high levels of genetic structure across their northern range. Furthermore, re-introduced populations were found to have slightly reduced levels of genetic variation relative to populations that have persisted without artificial augmentation. Only two re-introduced populations, however, revealed significantly lower levels of heterozygosity when compared to the nearest adjacent indigenous population (Table 4-1).

Genetic Variation in Indigenous Populations

Most fisher populations have undergone significant historical declines (Brander and Brooks 1973, Powell 1993). For example, in New York the number of fishers harvested between 1920 and 1950 decreased by 75% (DeVos 1952). If these drastic declines also reduced the effective population size (N_E), then a concomitant decrease in the levels of genetic variation would also be expected. These declines do not seem to have greatly impacted the levels of variation in fisher populations (heterozygosity, H_E, = 62%) relative to the levels of variation found in other carnivores (e.g. wolves, H_E= 63%, Roy et al. 1994; brown bears H_E= 68%, Paetkau et al. 1998; martens H_E= 66%, Kyle et al. 2000; wolverines H_E=63%, Kyle and Strobeck 2001).

Re-Introduced Populations

In the re-introduced populations we observe a decrease in the level of genetic diversity compared to adjacent indigenous populations. On Manitoulin Island, where 17 fishers were introduced from the Bancroft district of Ontario, the levels of genetic variation are significantly lower than values found for the immediately adjacent population of French River (Table 4-1). There is, however, a possibility that animals persisted on this island and that the re-introduced animals have had little or no effect on the genetic composition of the indigenous population. Hence, current levels of genetic variation may simply reflect the fact that the Manitoulin Island population is isolated from mainland populations and has a lower N_E.

In Nova Scotia, the total absence of fishers after 1922 is well documented (Dodds and Martell 1971; Bensen 1959). The re-introduction of fishers to both the central and southern regions of the province from 1947-1966 is most likely the original source of all fishers in these areas. These founding populations (central region N=70; southern region N=20) have led to lower levels of genetic variation relative to adjacent, indigenous populations in Quebec (Table 4-1). However, only the central Nova Scotia population was found to have significantly lower heterozygosity compared to the Rimouski population. The fact that a dramatic loss of H_E is not observed in these populations may suggest that Nova Scotia fishers originally came from populations with high levels of heterozygosity and/or that multiple re-introductions have maintained the level of H_E. Another possibility is a low level of gene flow between these populations.

The assignment tests suggest that all three re-introduced populations are quite insular with few cross-assignments to other populations. Manitoulin Island had 41/43 individuals assigned to itself. The two cross-assignments observed were both to French River. Central Nova Scotia had 38/41 individuals assigned to itself and the southern population had 15/17 individuals assigned to itself. Cross-assignment occurs between the Nova Scotia populations. This may suggest that a few individuals have dispersed from the site of their re-introduction. This is not unexpected as fishers are known to disperse relatively long distances from the region of re-introduction, depending on the time of year they are released (Proulx et al. 1994, central Alberta).

Both D_S and pairwise F_{ST} values (Table 4-2) suggest that the two sampled Ontario populations are quite similar genetically. It is not clear if the observed genetic distances and F_{ST} values are the result of genetic similarities between French River and Bancroft district animals (source of reintroduced animals to Manitoulin Island) or similarities to indigenous Manitoulin Island fishers.

We were unable to assess genetic differentiation between central Nova Scotia fishers (founded from Maine) and fishers from Maine as no animals were obtained from the source population. The relatively large genetic distance between Nova Scotia populations suggests that they may have different origins, or that a significant amount of genetic drift has occurred due to relatively small founding populations.

Population Genetic Structure of Indigenous Populations

The population genetic structure was assessed using a genotype assignment test, two genetic distance measures, and pairwise F_{ST} estimates (Table 4-2). Overall, the structuring observed across the sampled populations is consistent with isolation by distance (see Results). The levels of structure observed may be related to the fact that this species is thought to be relatively habitat sensitive and is not thought to disperse large distances from its maternal home range (Powell and Zielinski 1994). Furthermore, heavy snowfalls may limit the dispersal of fishers (Raine 1983; Aubry and Houston 1992).

An exception to isolation by distance was found among Alberta populations originally designated north, west, and central (pooled into Alberta west in tables) whose allele frequencies were found to be very similar. This finding may reflect the recent, rapid growth and radiation of fisher populations in these regions after having undergone considerable declines in the early to mid 1900's.

Population Genetic Structure Relative to Closely Related Mustelids

Although the results are not directly comparable, as many different loci were used and evolutionary rates of loci may differ in different species, the assignment test and the pairwise values of F_{ST}, D_{LR} and D_S, in general, suggest that fisher populations are more structured than other closely related mustelids (martens, Kyle et al. 2000; wolverines, Kyle and Strobeck 2001). In these studies an overall F_{ST} value of 0.0198 was observed in northern martens and a value of 0.0427 in wolverines. For fishers the overall F_{ST} was 0.1357. In both the northern marten study and in the northern wolverine populations, only 15% - 50% of individuals were assigned to the population from which they were sampled, as compared to 70-96% in fisher populations. Furthermore, calculating the slope from the linear regression of genetic versus geographic distance revealed that fishers have nearly two and five times more structure, per unit distance, than either northern martens or wolverines, respectively (see Results).

It is not surprising that wolverine populations have much less structure than fishers given their ability to disperse hundreds of kilometers in a short time (Magoun 1985) and their ability to cross potential physical barriers with relative ease (e.g. mountain ranges, large rivers, unsuitable habitat). The finding that martens revealed less genetic structuring than fishers did not fit our predictions. This unexpected result may lie in the different life history characteristics of these two species. Martens are thought to have limited dispersal ability (Buskirk and Ruggiero 1994). Fishers are able to disperse up to 60 km from their natal range (Leonard 1980; Raine 1987) with an average natal dispersal of about 10km (Arthur et al. 1993). With this in mind, the expectation would be that martens would have more structure than fishers. Martens, however, are not limited by heavy snowfall, as are fishers. Furthermore, fisher populations may be exposed to stronger anthropogenic influences (human development, transportation corridors, loss of suitable habitat) than the marten populations from the Yukon and Northwest Territories sampled in Kyle et al. (2000). These potential anthropogenic influences may act as barriers to dispersal for fishers. The combination of these factors may explain why fishers display much more structure than northern martens, although further study on the dispersal characteristics of this species in various environments will be needed to discern which influences have a greater impact on their population genetic structure.

Acknowledgements

I would like to thank all those who provided specimens for this study: Fraser Corbould and Rich Weir (Peace/Williston Fish and Wildlife Compensation Program); Bob McClymont, Floyd Kunnas, and Bruce Trichel (Alberta Fish and Wildlife), Barry Verbiwski (Manitoba Wildlife Branch), Neil Dawson (Ministry of Natural Resources), Pierre-Yves Collin and Jean Lamoureux (Société de la faune des parcs du Québec), Mike Boudreau, Mike O'Brien, and Doug Archibald (Nova Scotia Natural Resources), Marie Kautz and Mark Brown (New York State Department of Environmental Conservation), North American Fur Auctions (Wayne and Marlene Sharpe), and Alberta Trapper's Association (Maggie Boe and Fred Neumann).

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Table 4-1. Genetic variation as measured by: the mean number of alleles; mean, unbiased heterozygosity; unbiased probability of identity.

Population	Abbr	N	Mean # Alleles	%Het.	Probability of ID: 1 in
NS –central*	NSc	41	4.31	56.16	85, 300, 000
NS –southern*	NSs	17	4.00	57.58	112, 200, 000
QC –southeast	QCs	45	4.77	62.76	4,220,000,000
QC –Rimouski	QCr	38	4.77	66.66	17,200,000,000
NY –Adirondacks	NY	24	4.23	63.04	1, 554, 000,000
ON –Manitoulin I.*	ONm	43	4.62	63.20	1, 895, 000, 000
ON –French River	ONf	41	5.85	68.31	128, 500, 000, 000
MB –southeast	MB	21	5.54	68.11	128, 700, 000, 000
AB –east	ABe	22	4.15	61.82	1, 213, 000, 000
AB –west**	ABw	67	4.69	61.02	1,013, 000, 000
BC –Peace	BCp	16	4.00	62.00	578,000,000
BC –Omineca	BCo	66	4.54	58.45	208,000,000
BC –soutern interior	BCs	18	3.92	60.17	341,000,000
Average/Total		459	4.56	62.25	21,970,000,000

* Re-introduced populations

** AB-west represents the sampled regions Alberta north, central, and west pooled into one population.

Table 4-2. Genetic distances: Upper diagonal, Nei's standard distance, D_S. Lower diagonal, Pairwise F_{ST}.

	NSc	NSs	QCr	QCs	NY	ONf	ONm	MB	ABe	ABw	BCo	BCp	BCs
NSc	0	0.4	0.26	0.26	0.22	0.4	0.43	0.42	0.43	0.38	0.62	0.59	0.64
NSs	.192	0	0.32	0.32	0.23	0.36	0.49	0.45	0.6	0.47	0.55	0.6	0.75
QCr	.121	.128	0	0.12	0.17	0.3	0.42	0.29	0.35	0.31	0.41	0.42	0.44
QCs	.129	.138	.049	0	0.21	0.36	0.39	0.37	0.41	0.36	0.38	0.5	0.42
NY	.112	.102	.067	.089	0	0.22	0.26	0.26	0.39	0.29	0.4	0.44	0.53
ONf	.162	.141	.103	.131	.082	0	0.13	0.26	0.3	0.23	0.26	0.32	0.35
ONm	.188	.191	.152	.153	.108	.048	0	0.26	0.36	0.4	0.42	0.47	0.54
MB	.172	.165	.098	.132	.094	.028	.100	0	0.26	0.18	0.19	0.28	0.27
ABe	.192	.224	.130	.159	.152	.111	.147	.097	0	0.1	0.26	0.24	0.32
ABw	.178	.195	.127	.151	.128	.098	.164	.077	.046	0	0.12	0.12	0.17
BCo	.250	.226	.164	.165	.172	.117	.177	.085	.121	.064	0	0.16	0.1
BCp	.233	.223	.147	.181	.163	.116	.172	.100	.096	.050	.078	0	0.17
BCs	.250	.261	.159	.166	.194	.131	.197	.100	.134	.078	.045	.070	0

Note: AB-west represents the regions Alberta north, central, and west pooled into one population.

Figure 4-1. Map of sampled regions.

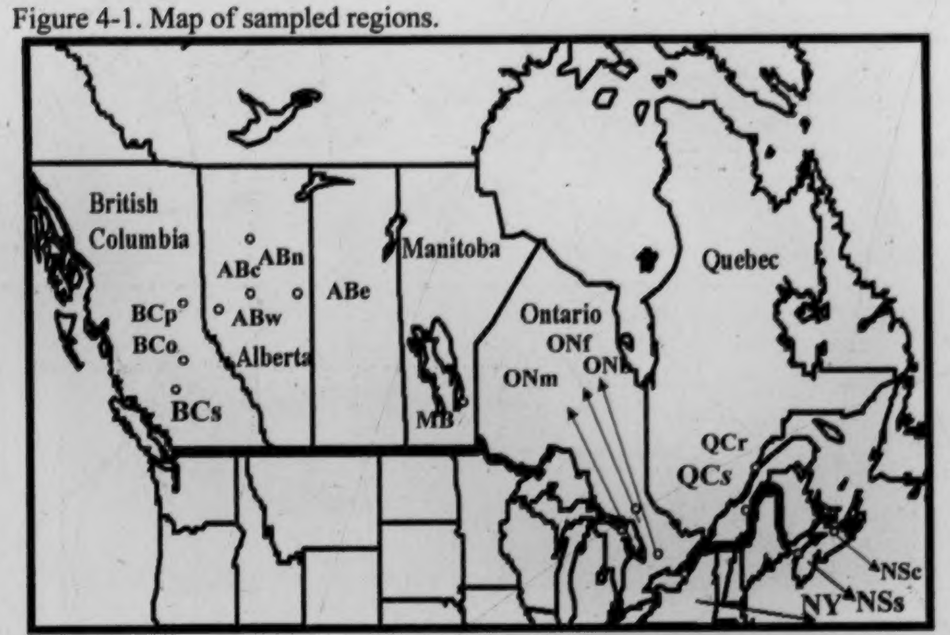
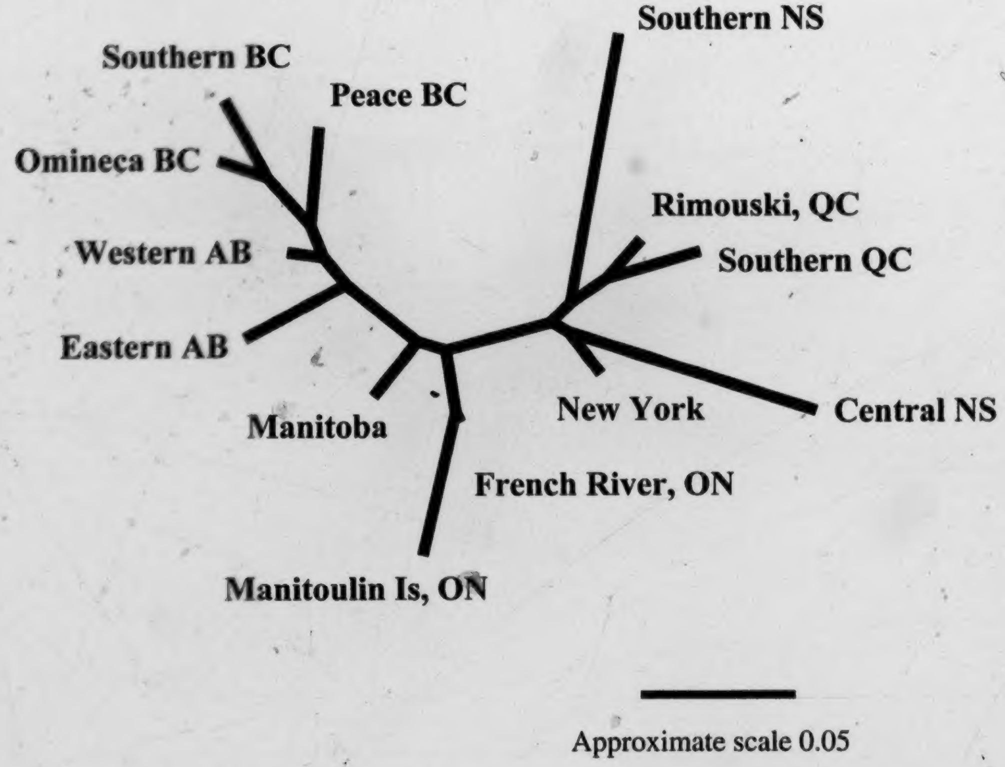


Figure 4-2. Unrooted neighbour-joining tree of pairwise F_{ST} values.



Chapter 5

Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories*

Introduction:

The American pine marten (*Martes americana*) is one of the most commercially important North American furbearers, both in terms of the quantity of animals harvested and the economic value of the harvest. With increasing concern about how this forest carnivore might be managed effectively, there is a need for genetic studies to discern the levels of effective migration between marten populations. Such data would be difficult to obtain by direct observation of this relatively elusive species. By elucidating the levels of gene flow which occur between populations we can ascertain how isolated populations are and what features - topographical, anthropogenic, or otherwise - influence the movement of individuals. Such information will provide a genetic basis with which to assess conservation strategies for this furbearer and potentially influence current trapping strategies and harvest quotas.

This study was undertaken to determine the levels of population genetic structure of martens across part of their northern distribution, from the Yukon Territory across to the central Northwest Territories (NWT). By describing the genetic structure of northern marten populations we are hoping to illustrate the levels of population subdivision in a relatively undisturbed and continuous habitat. This information can then be compared to the population genetic structure of more southerly populations where suitable marten habitat is more fragmented (see review by Buskirk and Ruggiero 1994).

Martens are found in all temperate to arctic zones spanning North America including many offshore islands (Hall 1981), however the majority of their distribution is in the boreal and taiga zones of Canada and Alaska (Buskirk and Ruggiero 1994). In Canada the only marten population given endangered status by COSEWIC (1998) is the potential subspecies *M. a. atrata* in Newfoundland, although other populations in Nova Scotia (including Cape Breton), and Prince Edward Island are thought to be endangered or extinct (Thompson 1991). Several life history traits of this species may have important influences on its population structure. Martens exhibit a large degree of sexual dimorphism, including not only morphological characteristics in males and female but also in their home-range size and potential for dispersal (Buskirk and Ruggiero 1994). The reported values for the average home range size of a Northern American pine marten in the more southern reaches of its distribution vary from 0.8 km^2 in Montana (Burnett 1981) to 15.7 km^2 in Minnesota (Mech and Rogers 1977), with female home ranges about half as large as those of males (Buskirk and Ruggiero 1994).

***A version of this chapter has been published. Kyle, C.J., C.S. Davis, and C. Strobeck, Canadian Journal of Zoology 78: 1150-1157, 2000.**

Furthermore, home ranges vary according to prey abundance and habitat type (Thompson and Colgan 1987). Densities of martens range from 0.4 to 2.4 animals per km^2 as reported by Thompson and Colgan (1987) for Ontario martens in times of prey scarcity and abundance, respectively. In the northern reaches of this species' range, densities of 0.6 animals per km^2 have been recorded (Archibald and Jessup 1984; Francis and Stephenson 1972).

Population fragmentation might be expected for martens due to its strong association with particular habitat types, which are themselves fragmented across certain landscapes. Significant population genetic structure has been found in wolverine populations (Kyle and Strobeck 1999), a closely related species with similar mating dynamics (Banci 1994). Martens have a relatively small home range size as compared to wolverines, and their potential for long distance dispersal is not as high, therefore the expectation is that marten populations may be even more genetically structured. Martens, however, exist in much higher densities than wolverines with 0.6 martens per km^2 recorded in the Yukon (Archibald and Jessup 1984; Francis and Stephenson 1972) versus 5.6 wolverines per 1000 km^2 (Banci and Harestad 1990). Hence, much larger population sizes may contribute to less population genetic structure being observed, especially if migration rates among local populations are high.

As with many other mustelid species, studies of population genetics for the American pine marten are scarce and of limited scope. Hicks and Carr (1991) investigated genetic structure in Newfoundland martens using a section of cytochrome b of mtDNA, but small sample sizes did not allow for clear resolution of the levels of gene flow among these populations. A second study by Mitton and Raphael (1990) examined ten martens from Wyoming and screened 24 allozyme loci revealing only 17% heterozygosity. A more recent study by McGowan et al. (1999) used RAPDs to try and estimate the levels of heterozygosity among four marten populations sampled from Newfoundland, Labrador, and two other Canadian regions. The average heterozygosity was found to range from 0.026 to 0.226. With the wealth of ecological information that exists for this species there is a need to complement previous ecological work with statistically significant population genetic studies to make relative assessments of genetic variability and gene flow across North America.

To detect genetic differentiation among marten populations, high resolution (fast evolving) neutral genetic markers are preferred. For this reason, we have chosen to use hypervariable microsatellite loci. These polymorphic, tandem repeats of DNA have proven useful in other studies of mammalian species with high vagility, such as the polar bear (*Ursus maritimus*) (Paetkau *et al.* 1995) and North American brown bear (*Ursus arctos*) (Paetkau *et al.* 1998). Microsatellites identified clear genetic differentiation in these animals despite the long-range movements known to occur in these species, and the fact that little variation was detected using other methods.

Sampled Areas and Methods:

Twelve areas were sampled for *Martes americana* in the Yukon and NWT (Figure 5-1). Samples were obtained from fur harvesters and collected by conservation agencies in each of the respective regions. The NWT samples were collected from: Fort Providence (Ft.Prov.), Fort Simpson (Ft.Simp.), Déliné, Turton Lake (Turton), Fort Good Hope (Ft. G. H.), Fort Resolution (Ft. Res.), and Fort Smith (Ft. Smith). With the exception of the Turton Lake samples, which came in the form of hairs, all NWT samples were from frozen muscle tissue. The Yukon populations were collected from particular traplines (lines 17, 23, 26 near Dawson City; line 314 east of Whitehorse; and line 365 near Watson Lake) with all samples in the form of bone, some with small parts of dried tissue remaining on them.

The taxonomic status of North American pine martens is somewhat controversial. On the basis of morphological characters as many as six distinct species have been described (Miller 1923) and fourteen subspecies (Hall and Kelson 1959). Carr and Hicks (1995) suggest that there are only two distinct species; *Martes caurina*, found in the southwestern region of the species distribution and, *Martes americana*, found across the remaining North American range from Alaska to Newfoundland. One mitochondrial DNA haplotype characterizes *M. americana* across this entire range. The species distinction between *M.a.caurina* and *M.a.americana* is, however, based on only 1.6% sequence variation between the two groups in a 401 bp region of the cytochrome b gene in mitochondrial DNA. According to Carr and Hicks (1995), within these two groups seven subspecies are found within *M. americana* and six within *M. caurina* (one remaining subspecies of the fourteen thought to exist in North America, *M. a. humboldtensis*, has yet to be studied). The animals used in this study are all from within the *M. americana* distribution as per Carr and Hicks (1995).

DNA was extracted from all samples using a QIAamp® Tissue Extraction Kit. All specimens were run at twelve microsatellite loci (MA-1, MA-2, MA-3, MA-7, MA-8, MA-9, MA-10, MA-11, MA-14, MA-15, MA-18, and MA-19) which were previously developed in martens (Davis and Strobeck 1998). Amplification of DNA at all loci was performed using the same protocol as found in Davis and Strobeck (1998). The DNA fragments were then visualized using an ABI Prism™ 377 DNA sequencer. Analysis of DNA fragments was done using the programs GeneScan™ Analysis 2.02 and Genotyper® 2.0.

Data Analysis

The first step in the analysis of the data was to determine if the loci used to elucidate the population genetic structure amongst these geographical areas conformed to Hardy-Weinburg equilibrium (H.W.E.) by testing for both heterozygote excess and deficit. This was assessed using Genepop version 3.1 (Raymond and Rousset 1995) which uses a Markov chain method following the algorithm of Guo and Thompson (1992). A *G*-test for heterogeneity (Sokal and Rohlf 1995) was then performed for each of the sampled areas by making pairwise comparisons of allele distributions. Genepop was also used to provide an overall F_{st} estimate (as per Weir and Cockerham 1984).

The relative genetic variation in each population was first assessed using allele frequency data from which the mean number of alleles, unbiased expected heterozygosity (Nei and Roychoudhury 1974), and unbiased overall probability of identity (Paetkau et al. 1998) were calculated. The genetic distance between the populations were estimated using two measurements: Nei's standard genetic distance, (D_S, Nei 1972) which is calculated from genotype frequencies and the genotype likelihood ratio, (D_{LR}, Paetkau et al. 1997) which is calculated from genotype probabilities. Both sets of genetic distance values were calculated by programs within the website http://www.biology.ualberta.ca/jbrzusto/alpha/Doh/html, designed by John Brzustowski. An unrooted Neighbor joining tree of the D_S values was then created using PHYLIP 3.572 (Felsenstein 1995). The geographic and genetic distance values were also entered into a two-way Mantel test within the set of programs called the "R" Package for mulitvariate analysis designed by Alain Vaudor (Mantel 3.0; Mantel 1967) to determine the correlation between genetic distance and geographic proximity.

The assignment test (Paetkau *et al.* 1995), also found on the aforementioned web site by John Brzustowski, was run for all populations. This program determines both the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in the regions to which it is being compared. It then assigns individuals to the population in which that individual's genotype has the highest probability of occurring (see Waser and Strobeck 1998). The assignment test was run in several different ways, using the following options: replacement of gene frequency values of 0 with 0.01, resampling (2000 iterations) of the populations from within each gene pool assuming H.W.E., and resampling (2000 iterations) of the populations by combining the gene pools. The option of resampling the data from each gene pool provides a significance value for the assignment of individuals to particular populations. Each replicate generated by the randomization option uses allele distributions observed within the population to generate an equal number of new genotypes. Of the number of randomization tests performed (2000 in this case), the number of replicates that are equal to or greater number of cross-assignments are noted (R). Cross-assignments that occurred with a frequency of $R/2000 \leq 0.05$ were considered significant, and were used to infer the presence of migrants. The presence of migrants is inferred since as great a number of cross-assignments as observed would not be expected to occur by chance. A population that has no cross-assignments would be expected to have a frequency of $R/2000 = 1.0$ replicates that have an equal or greater number of cross-assigned individuals. This option of randomizing the data from each gene pool is particularly useful when there are distinct regions with little gene flow between them when trying to identify the source of the potential migrants between regions. Conversely, all that can be said of cross-assignments that are found to be significant (by the randomization of individuals within each gene pool) between regions that do not have strong breaks in gene flow is that there is a lot of migration between the regions. It should therefore be noted that cross-assignments are not necessarily migrants from one population to another. When the option of combining the gene pools is used, the values obtained suggest how heterogeneous the populations are using genotype probabilities as opposed to genotype frequencies as in the G-test for heterogeneity.

The genotype assignment test was also run with only females included and then only males in an attempt to distinguish between male and female dispersal amongst the populations. It should also be noted that complete genotypes were not obtained for two individuals from Yukon line 365 and three for Yukon line 26. For this reason these five individuals were not included in any of the assignment test analyses.

Results:

The twelve geographic locations sampled in this study (Fig. 5-1) were analyzed at twelve microsatellite loci. A *G*-test revealed that all sampled regions were genetically heterogeneous despite the close proximity of some populations to one another. The locus MA-15 was found to deviate from H.W.E. in all populations due to an excess of homozygotes, most likely caused by presence of null alleles. For this reason, MA-15 was then dropped from further analyses and none of the data shown includes information from this locus. Of the 121 genotype distributions, a heterozygote deficit was found for 22 of these and an excess for only one at the 5% level. However, when the Dunn-Sidak experiment-wise error rate was used (Sokal and Rohlf 1995), only two genotype distributions deviated from H.W.E. at the 5% level. Both traplines 23 at locus MA-6 and 26 at locus MA-2 showed heterozygote deficits.

The mean number of alleles ranged from 5.18 in the Turton Lake population to 6.64 in the Yukon trapline 314 population (Table 5-1). A trend of higher values for Yukon regions compared to NWT regions was observed, but this was most likely associated with an increase in sample size for the Yukon populations. The mean heterozygosity values were relatively homogeneous across the sampled regions, ranging from 61.7% at Fort Good Hope to 67.5% at Yukon trapline 17. There was a little more variation in the unbiased probability of identity estimates, which ranged from 1/984 million at Fort Good Hope to 1/29.6 billion at Fort Smith.

The population assignments, randomizations from each gene pool, and randomizations grouping the gene pools results from the assignment test are summarized in Table 5-2 (a and b respectively). Fort Providence had 9/30 individuals correctly assigned to itself with 5 individuals assigned to Deliné. The remaining cross-assigned individuals were distributed relatively evenly among the other sampled regions. Fort Simpson had 14/30 individuals correctly assigned to itself, again with the cross-assigned individuals evenly distributed among the other regions. Both Déliné and Turton Lake few individuals correctly assigned to the population from which they were sampled with only 7/30 and 3/16 individuals correctly assigned to them, respectively. Fort Good Hope had 16/30 individuals correctly assigned to itself with the remainder of individuals sampled from that region cross-assigned evenly amongst the other regions. Fort Resolution had 22/30 individuals correctly assigned to itself whereas Fort Smith had 19/30 individuals correctly assigned. The Yukon traplines 314 and 365 had 14/40 and 10/36 individuals correctly assigned, respectively. The Yukon traplines 26, 23, and 17 had only 3/37, 8/31, and 6/40 individuals correctly assigned, respectively. The majority of the cross-assignments for these three traplines were to the other traplines in the same region.

The randomizations of the individual genotypes from the individual gene pools (Table 5-2 (b)) could not distinguish migrants because no distinct areas or breaks in the level of gene flow were found amongst these populations. A trend that can be seen in Table 5-2b is across the diagonal of randomized assignments where the number of assignments are much higher than expected by chance. This suggests that there is a high level of migration into each of the populations.

The randomizations of the individual genotypes from the combined gene pools suggests that the Turton Lake is, genetically, not strongly separated from Fort Good Hope. These populations are only separated by about 30 km. The Yukon traplines 26, 23, and 17 were not strongly structured populations as shown by the randomization of the combined gene pools. This follows from the low percentage of individuals correctly assigned to these populations (Table 5-2 (a)). Fort Providence does not seem to be strongly segregated from Déliné, this is surprising when taking into account of their geographic separation of about 500 km. An overall estimate of population structure was calculated using Genepop version 3.1 (Raymond and Rousset 1995) which revealed a relatively low F_{st} estimate (as per Weir and Cockerham 1984) of 0.0198.

The genetic distance measurements D_S and D_{LR} were both correlated to geographic distance as determined by a two-way Mantel test (Mantel 1967). The correlation coefficient, r, was 0.203 when geographic distance was compared to D_{LR} ($p = 0.10$), and $r = 0.264$ ($p = 0.035$) when compared to D_S. The Unrooted Neighbor Joining trees of both measures of genetic distance illustrate the isolation by distance correlation from the Mantel test. Geographically proximate populations are, for the most part, also closest genetically, with the exception of Fort Resolution and Fort Smith which were further removed from the other populations. Turton Lake and Fort Good Hope are strongly linked as are the Yukon traplines 314 and 365. Finally, the Yukon traplines 26, 23, and 17 are relatively close together both genetically and geographically.

The assignment test was also run by segregating out both males and females to determine if males showed less structure than females due to their expected sexual dimorphism in dispersal characteristics. No evidence could be found to suggest that this was the case.

Discussion:

The levels of genetic variation across the sampled region in this study seem to be relatively homogeneous (Table 5-1). Most variation in the levels of diversity can be attributed to the number of individuals sampled in a particular population. The results of this study are similar to that of other carnivore population genetic studies (Kyle and Strobeck 1999; Paetkau et al. 1998) in that the northern distributions of these species' do not seem to be fragmented. In northern wolverine populations sampled from western Alaska through to central Nunavut, the levels of genetic variation and migration were relatively high as compared to more southerly populations in Southern British Columbia and Idaho (Kyle and Strobeck, 2000, submitted). This trend can also be seen in brown bears (Paetkau et al. 1998) where the northern populations were less fragmented and estimates of genetic diversity were higher than in the southerly reaches of its distribution.

The assignment test results, summarized in Table 5-2 (a and b), show that there is some significant structure among the populations, but most of the populations are not highly structured. Turton Lake, and Yukon traplines 26 and 17 did not show significant structure according to the resampling of individuals from the combined gene pools (Table 5-2). This is most likely due to the fact that Turton Lake is very close to Fort Good Hope and the Yukon traplines 17, 23, and 26 are in very close proximity to one another, hence there may be considerable gene flow among these regions. Fort Resolution and Fort Smith both had about 2/3 of the individuals correctly assigned, and Fort Simpson and Fort Good Hope had about 1/2 of the individuals correctly assigned. The remaining populations, however, had 1/3 or less of the individuals correctly assigned to the population from which they were sampled. This would suggest that there is a lot of movement amongst marten populations. The genetic homogenization of the northern marten populations may be caused by high rates of short-distance migration across a continuous landscape as seen in Columbian ground squirrels using allozyme data (Dobson 1994). The assignment test results, however, suggests that some long-distance migration is taking place, which in itself may explain the low levels of population genetic structure in this species. It should also be noted that the usefulness of the randomizations of the data from each gene pool for the identification of migrants, in this study, was diminished due to the lack of highly structured populations. Had the populations been highly structured, so that genotypes were more endemic to a particular area, it would have been possible to identify not only migrants into a population, but from which populations they migrated. In this particular case, we are only able to say that certain individuals did not seem to match the other genotypes from the population from which they were sampled, and that they seem to be more similar to those genotypes found in the population to which they were cross-assigned. We were unable to distinguish between male and female dispersal, any segregation between males and females most likely being diluted out by the mating of dispersing males. Our sampling design, however, did not allow us to test for high levels of localized population genetic structure that may be found in species where males are the primary dispersers, such as in most mustelid species (Nunney 1999).

The measurements of genetic distance, D_{LR} (from genotype probabilities) and D_S (from allele frequencies) present similar results (Table 5-3 (a) and (b)) to one another. The most proximal regions have the lowest values of genetic distance, with the exception of Fort Resolution and Fort Smith. The only inconsistency between the two unrooted Neighbor Joining trees of genetic distance (Figures 5-2 and 5-3) is the association of the Déline population with Turton Lake and Fort Good Hope on the D_{LR} tree, whereas it is more closely associated with Yukon traplines 314 and 365 on the D_S tree. When the correlation between geographic distance and the genetic distance was investigated using a Mantel test, a significant, although weak, correlation was observed for the D_S values. A nearly significant correlation was observed for the D_{LR} values.

This study reveals the genetic structure of marten populations in the northern reaches of their distribution. The structure seems to be mainly due to isolation by distance and not population fragmentation in the regions sampled as might be expected in more southerly regions where habitat is more fragmented. The lack of genetic structure suggests a high level of gene flow among the regions sampled and relatively large population sizes. This is in following with the estimated density of this species, 0.6 animals per km^2, recorded in the Yukon (Archibald and Jessup 1984; Francis and Stephenson 1972) and the potential for martens to disperse over relatively large distances (Archibald and Jessup 1984).

More population structure was expected between the Yukon and NWT, however, with potential barriers to gene flow such as the physical barriers of the MacKenzie mountains, Great Slave Lake and Great Bear Lake. These results suggest that even large mountain ranges have little effect on gene flow between marten populations. The most distinct populations of Fort Smith and Fort Resolution are found on the southeast side of Great Slave Lake. This lake may act as a physical barrier to gene flow, although these two populations did not group together on either genetic distance tree. It should be noted that both populations are found on the border of the taiga shield/taiga plains ecozones. Further investigation of populations from within the taiga shield ecozone will be needed to reveal if a genetically distinct group of martens exists within this ecozone. Further analysis of southern marten populations will also investigate if southerly populations are fragmented by a less continuous habitat and human encroachment as are other carnivore species such as wolverines and brown bears (Kyle and Strobeck 1999; Paetkau et al. 1998).

Acknowledgements:
I would especially like to thank those people who collected and made the samples available for this study including: all the trappers that provided samples, Kim Poole and Alasdair Veitch (NWT Renewable Resources) and in the Yukon Territory, Brian Slough and Philip Merchant (Yukon Renewable Resources).

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Table 5-1. Genetic variation: mean number of alleles, mean heterozygosity, and overall unbiased probability of identity of *M. americana* using 11 microsatellite loci.

	(N)	# alleles	Heter.	Prob. I.D. 1/
Ft. Prov	30	5.55	64.3	7,230,000,000
Ft. Simp	30	5.91	67.2	17,980,000,000
Deliné	30	6.00	64.7	4,450,000,000
Turton	16	5.18	66.5	7,230,000,000
Ft. G.H.	30	5.36	61.7	984,000,000
Ft.Res.	30	5.27	63.8	2,850,000,000
Ft.Smith	30	6.00	66.2	29,600,000,000
YK(314)	40	6.64	67.1	10,590,000,000
YK(365)	38	6.09	65.5	6,850,000,000
YK(26)	40	6.27	66.1	7,280,000,000
YK(23)	31	6.09	66.7	13,140,000,000
YK(17)	41	6.27	67.5	14,830,000,000

Table 5-2. Population assignments from the Assignment Test. The population list in the left margin indicates where the individuals were sampled from and the top list is the population to which the individuals were assigned to with the highest probability.

A) Number of individuals assigned to each population.

	(N)	Ft. Prov	Ft. Simp	De-liné	Tur-ton	Ft. G.H.	Ft. Res.	Ft. Smit	YK (314)	YK (365)	YK (26)	YK (23)	YK (17)
Ft. Prov	30	**9**	2	5	2	2	2	0	3	2	0	2	1
Ft. Simp	30	3	**14**	1	2	2	1	1	0	1	0	1	4
Deliné	30	1	2	**7**	4	3	0	0	3	2	3	3	2
Turton	16	2	0	2	**3**	4	0	0	1	0	1	1	2
Ft. G.H.	30	3	1	3	2	**16**	0	0	0	3	1	0	1
Ft.Res.	30	3	2	1	0	0	**22**	0	0	0	1	1	0
Ft.Smith	30	2	1	0	1	0	1	**19**	2	3	0	0	1
YK(314)	40	0	2	2	3	2	0	2	**14**	4	2	6	3
YK(365)	36	5	2	2	0	5	0	5	3	**10**	2	0	4
YK(26)	37	0	2	2	5	1	1	0	2	1	**3**	10	10
YK(23)	31	0	1	2	3	1	1	1	1	1	8	**8**	4
YK(17)	40	3	2	2	4	2	1	1	3	4	8	4	**6**

B) Randomization of assignment test (2000 iterations) sampling from each gene pool and assuming Hardy-Weinberg equilibrium. Bold indicates significant values.

	Ft. Prov	Ft. Simp	De-liné	Tur-ton	Ft. G.H.	Ft. Res.	Ft. Smit	YK (314)	YK (365)	YK (26)	YK (23)	YK (17)
Ft. Prov	1989	743	**107**	1036	1179	263	2000	**114**	1051	2000	297	1336
Ft. Simp	615	1851	1492	351	813	725	902	2000	1591	2000	1081	**114**
Deliné	1859	692	1985	291	549	2000	2000	325	1024	591	373	925
Turton	784	2000	764	1997	**54**	2000	2000	610	2000	1385	708	396
Ft. G.H.	634	1203	349	963	1837	2000	2000	2000	**97**	1225	2000	1299
Ft.Res.	**65**	**90**	711	2000	2000	1992	2000	2000	2000	717	633	2000
Ft.Smith	1138	1077	2000	773	2000	480	1707	295	246	2000	2000	1330
YK(314)	2000	1263	1358	253	1073	2000	556	1947	1190	1076	**25**	624
YK(365)	261	1154	1335	2000	**15**	2000	**10**	1381	1985	1001	2000	474
YK(26)	2000	711	1421	**167**	1616	1177	2000	884	1681	2000	**12**	**15**
YK(23)	2000	1257	911	**132**	1325	779	931	1544	1401	**40**	1997	318
YK(17)	704	1330	1378	297	1298	869	1469	638	636	**126**	553	1998

Table 5-3. Genetic Distances: a) D_{LR}; b) Nei's Standard Distance (D_S) and c) Geographic Distances

a) D_{LR} values:

	Ft. Prov	Ft. Simp	De-liné	Tur-ton	Ft. G.H.	Ft. Res.	Ft. Smit	YK (314)	YK (365)	YK (26)	YK (23)	YK (17)
Ft. Prov	0											
Ft. Simp	0.74	0										
Deliné	0.28	0.79	0									
Turton	0.33	1.13	0.18	0								
Ft. G.H.	0.75	1.21	0.79	0.57	0							
Ft.Res.	2.21	2.6	2.31	2.71	2.88	0						
Ft.Smith	1.04	1.73	1.36	1.75	1.95	3.43	0					
YK(314)	0.92	0.83	0.60	1.04	1.20	2.95	1.60	0				
YK(365)	0.53	0.76	0.46	1.00	1.28	2.80	1.27	0.25	0			
YK(26)	0.76	0.97	0.27	0.25	0.97	2.20	1.69	0.69	0.60	0		
YK(23)	1.16	1.19	0.59	0.88	1.20	2.42	1.70	0.79	0.93	0.01	0	
YK(17)	0.67	0.73	0.39	0.42	0.91	2.59	1.24	0.60	0.41	-0.03	0.36	0

b) Nei's Standard Distance (D_S)

	Ft. Prov	Ft. Simp	Deliné	Turton	Ft. G.H.	Ft. Res.	Ft. Smit	YK (314)	YK (365)	YK (26)	YK (23)	YK (17)
Ft. Prov	0											
Ft. Simp	0.05	0										
Deliné	0.05	0.06	0									
Turton	0.07	0.10	0.06	0								
Ft. G.H.	0.06	0.08	0.07	0.07	0							
Ft.Res.	0.10	0.09	0.11	0.15	0.13	0						
Ft.Smith	0.06	0.07	0.08	0.13	0.10	0.15	0					
YK(314)	0.07	0.06	0.04	0.08	0.07	0.13	0.08	0				
YK(365)	0.05	0.05	0.04	0.08	0.07	0.12	0.07	0.03	0			
YK(26)	0.07	0.07	0.04	0.06	0.06	0.11	0.11	0.05	0.05	0		
YK(23)	0.09	0.08	0.05	0.08	0.08	0.13	0.11	0.05	0.06	0.03	0	
YK(17)	0.05	0.05	0.05	0.06	0.06	0.12	0.08	0.04	0.04	0.02	0.04	0

Figure 5-1. Map of regions sampled for *M, americana.*

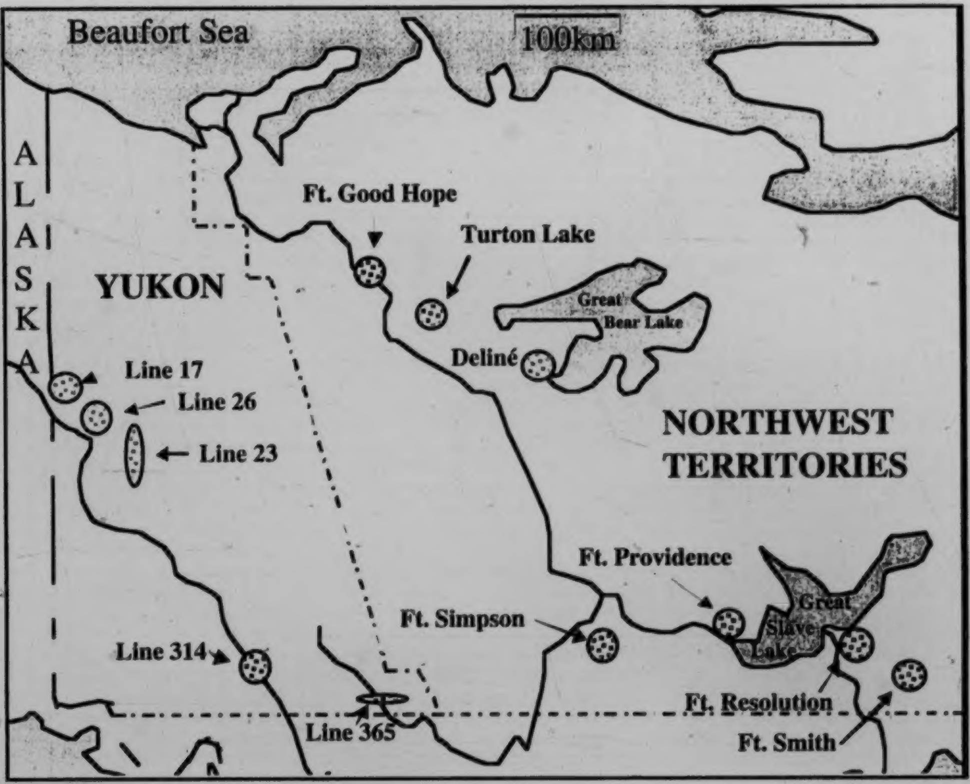


Figure 5-2. Tree of Nei's standard genetic distance.

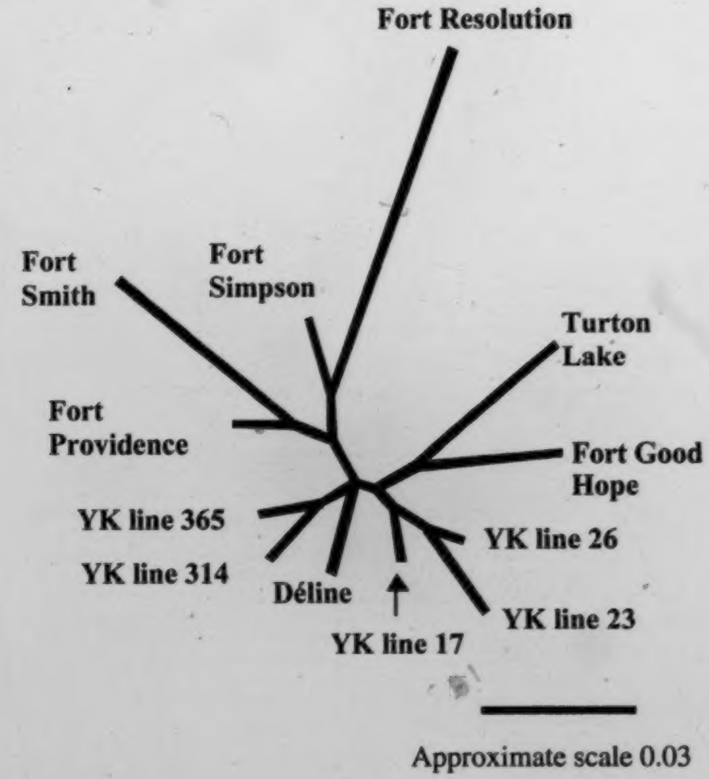
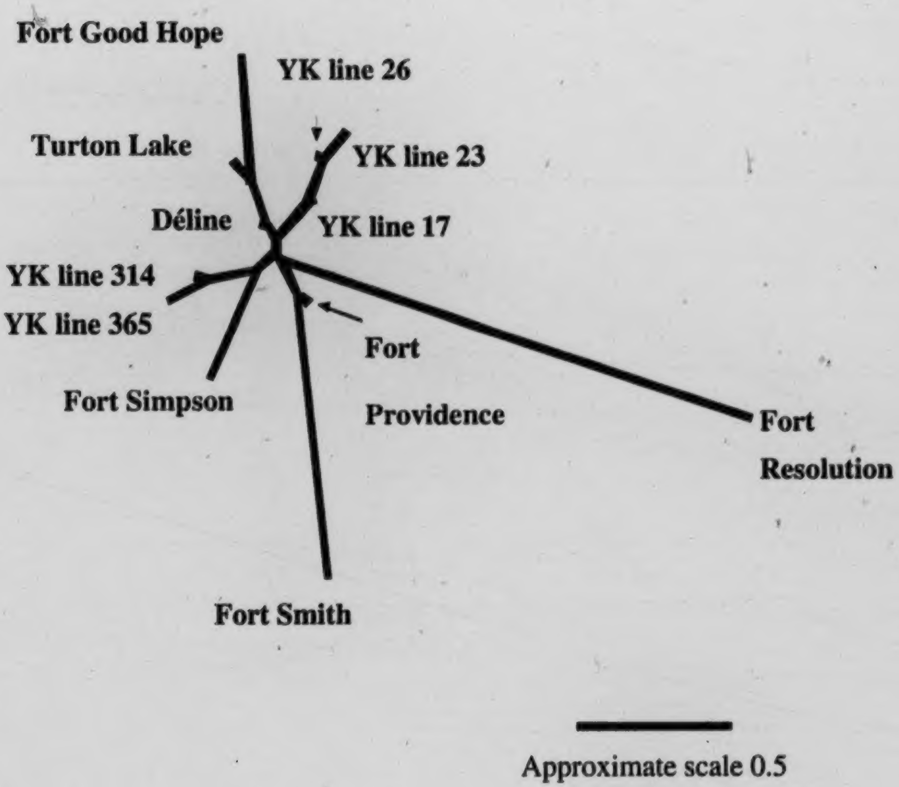


Figure 5-3. Tree of likelihood ratio distances.



Chapter 6

Genetic homogeneity of Canadian mainland marten populations underscores genetic distinctiveness of Newfoundland pine martens *(Martes americana atrata)**

Introduction:

American martens (*Martes americana*) are mid-sized mustelids that exist in the boreal and taiga zones of North America (Hall 1981). This species is thought to prefer late-successional coniferous forests (Allen 1987; Buskirk and Powell 1994; McLauren et al. 1998), although some challenge this generalization (Bowman and Robitaille 1997; Potvin et al. 2000; Mowat 2002). The combination of habitat loss due to intense logging practices, and extensive long-term fur harvests may be responsible for significant population declines and the extirpation of this species from several regions (Bissonette et al. 1989; Thompson 1991). Conservation efforts and forest succession have permitted the resurgence of martens in some regions, but their numbers are still considered low in areas such as the southern Rocky Mountains (Litvaitis 1993; Buskirk and Ruggiero 1994) and Maritime regions of Canada (Gibilisco 1994; Thompson 1991).

The effect of habitat fragmentation and intense harvesting on the genetic structure of marten populations is unclear. Habitat fragmentation has been shown to have a negative effect on martens, as fewer martens are captured or observed in clear-cut areas (Hargis et al.1999; Payer and Harrison 2000; Potvin et al. 2000; Forsey and Baggs 2001). Furthermore, tracts of treeless land (natural or anthropogenic) greater than 5 kilometers may act as complete barriers to marten dispersal (Hawley and Newby 1957; Gibilisco 1994). Martens also tend to avoid roads (Alexander and Waters 2000; Robitaille and Aubry 2000), which may therefore inhibit dispersal as well. However, martens in Banff National Park were found to use drainage culverts to cross the Trans-Canada highway (Clevenger et al. 2001).

With all of the aforementioned factors potentially impeding marten movement, we might expect marten populations to be as fragmented as the habitat in which they are said to associate. If this were the case, decreased levels of migration between regions would lead to smaller effective population sizes that would in turn lead to an increase in genetic structure, and a decrease in genetic variation due to drift. Such populations may be more susceptible to local extinction (Soule and Mills 1998; Gilpin and Soule 1986) and potentially lead to the loss of genetic variants that may be better adapted to future selective pressures.

Genetic variation and structure of American pine martens has been investigated using various molecular markers. Mitton and Raphael (1990)

***A version of this manuscript has been submitted to the Canadian Journal of Zoology (Kyle, C.J. and C. Strobeck, April 25, 2002).**

used allozymes to study 10 martens from Wyoming and found an average heterozygosity (H_E) of 17%. Hicks and Carr (1991,1997) used direct sequencing to compare the mitochondrial DNA (mtDNA) cytochrome b gene of martens from Newfoundland and mainland populations. A single mtDNA genotype was found for Newfoundland and all mainland populations east of the Rocky Mountains. McGowan et al. (1999) used randomly amplified polymorphic DNA (RAPD) markers to elucidate the genetic structure among four marten populations from British Columbia, Northwest Territories, Labrador, and western Newfoundland (island). They suggested that two clades of martens exist that are divided by the Rocky Mountains. Microsatellites have also been used to investigate the genetic structure of marten populations in the Canadian Territories (Kyle et al. 2000) where little genetic structure was observed despite vast distances separating some populations. In the relatively continuous habitat found in much of the Canadian Territories, isolation by distance was assumed to be the only determinant of population structure.

In contrast to American pine martens in the Canadian north, European pine martens (*M. martes*) from central and northwestern Europe, where the landscape has been heavily modified for centuries, were found to be highly structured (Kyle et al. submitted). This result was attributed to long-term habitat modification of European forests and the decrease in population sizes from harvest, poisoning, and sport hunting.

In the Canadian provinces, late-successional boreal forests have been exploited more recently than in Europe, and anthropogenic influences that may fragment marten habitat (forestry and road density) are more pronounced than in the Canadian north. Hence, an intermediate level of structure might be expected for marten populations in the Canadian provinces.

The goals of this study were threefold: (a) to elucidate the genetic structure of marten populations across Canada; (b) to compare populations found in relatively disturbed habitats in the Canadian provinces to that of relatively undisturbed habitats in the Canadian Territories; and (c) to compare the genetic structure of American pine martens to closely related mustelids and other carnivores to identify trends among species.

Sampled areas and methods

Samples were collected from areas throughout Canada including: western Newfoundland (island); Mingan (furbearer region or UGAF (Unités de gestion des animaux à fourrure 61), Port Cartier (UGAF 59), Godbout (UGAF 57), Forestville (UGAF 54), Eastmain, Waskaganish, and Gatineau (east and west sides of the Gatineau River) regions of Quebec; Pembroke, Chapleau, and Red Lake regions of Ontario; Cross Lake and Lac du Bonnet regions of Manitoba; Fort McMurray, High Level (traplines 1246 and 2273), Whitecourt (trapline 365), Edson (trapline 1257), and Nordegg (trapline 2259) regions of Alberta; and Slocan (see Mowat and Paetkau 2002 for details on sample collection), Golden, Prince George, and Chetwynd regions of British Columbia. This study also included data from Kyle et al. 2000, with samples collected from the Yukon (traplines 17, 23, 26, 314, and 365) and Northwest Territories (Fort Good Hope, Fort Resolution, Fort Providence, Fort Simpson, Fort Smith, Turton Lake, and Deline) (see Figure 6-1.). Samples were collected from fur auction houses, trapper's associations, or provided by federal and provincial wildlife agencies. Sample types included hair, hair from glue patches (see Mowat and Paetkau 2002 for details), pelt, bone, and muscle tissue.

Factors that may confound the true origin of individual samples are the numerous, and sometimes poorly documented, re-introduction efforts that have taken place over the last century. In Canada, there is documented evidence of marten re-introductions in Newfoundland, New Brunswick, Nova Scotia, Ontario, Manitoba, Saskatchewan, and the Yukon (see Van Zyll de Jong 1969; Slough 1994), but not in regions where our samples were collected.

DNA extraction and amplification

DNA was extracted from all sample types using a DNAeasy® tissue extraction kit (QIAGEN). Eleven polymorphic microsatellites were amplified using primers and conditions developed by Davis and Strobeck (1998) including: MA1, MA2, MA3, MA7, MA8, MA9, MA10, MA11, MA14, MA18, and MA19. Locus MA15 was found to contain many null alleles by Kyle et al. (2000) and so this marker was not amplified in any of the additional samples collected. DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer and the programs Genescan™ Analysis 2.02 and Genotyper® 2.0.

Data analyses

A G-test for heterogeneity of allele distributions, averaged across all loci (Sokal and Rohlf 1995), was performed for each pair of sampled areas. Departures from Hardy-Weinberg equilibrium (HWE) were tested for each of the eleven loci using Genepop 3.1d (Raymond and Rousset 1995). Genepop was also used to evaluate genotypic disequilibria among loci.

Relative genetic variation in each population was assessed using mean numbers of alleles (A), unbiased expected heterozygosity (H_E -Nei and Roychoudhury 1974), and the unbiased probability of identity (P_{ID} -Paetkau et al. 1997). Pairwise genetic distances were estimated using Nei's standard genetic distance (D_S -Nei 1972), based on allele frequencies, and the genotype likelihood ratio distance, D_{LR} (Paetkau et al. 1997), based on genotype probabilities. Both genetic distances were calculated by programs within the website: http://www.biology.ualberta.ca/jbruzsto/Doh/php. Pairwise F_{ST} estimates were obtained from Genepop (as per Weir and Cockerham 1984).

An unrooted neighbor-joining tree of D_S values (Fig. 6-2) was created using PHYLIP 3.573 (Felsenstein 1995). Pairwise genetic distances, F_{ST}, and geographic distance values were also entered into two-way Mantel tests (Mantel 1967) with programs obtained from the website:

http://www.fas.umontreal.ca/BIOL/legendre/, to test the correlation of the various measures and for isolation by distance.

Assignment tests were performed (Paetkau et al. 1995) using programs from the website: http://www.biology.ualberta.ca/jbruzsto/Doh/php. This program determines both the probability of a genotype occurring in the region from which it was sampled and the probability that it would arise in each of the other regions included in the test. Individuals are then assigned to the population where their genotype has the highest probability of occurring (see Waser and Strobeck 1998).

Results

After accounting for sample-wise error (Dunn-Sidak method; Sokal and Rohlf 1995), five departures from HWE were detected: MA1 in Prince George; MA7 in Gatineau; MA8 and MA19 in Chetwynd; and MA10 in Golden. Only locus MA7 in Chetwynd was found to deviate from HWE due to an heterozygote deficiency, and may suggest null alleles are present at this locus in this population. Genotypic disequilibrium was detected in three regions: Newfoundland with MA9/MA14; Gatineau with MA2/MA8; and Chetwynd with MA1/MA10. As no loci deviated from HWE in more than one population, and no pair of loci display genotypic disequilibrium in more than one locus pair in any given region, all loci were retained for analyses.

Due to the broad sampling scheme, large number of regions sampled, low pair-wise genetic distances and F_{ST} values, and for ease of comparison, all adjacent regions that did not differ significantly in their genotypic frequencies ($\alpha = 0.01$) were pooled (c.f. Kyle et al. 2000 where $\alpha=0.05$). All other regions were considered discrete populations for subsequent analyses. In the Yukon, traplines 17, 23, and 26 were pooled into the Dawson (DC) population, and traplines 314 and 365 were pooled as Watson Lake (LR). In the Northwest Territories, the regions Deline, Turton Lake and Fort Good Hope were all pooled into a population called Fort Good Hope (FG). In Alberta, traplines 1246 and 2272 near High Level were pooled as High Level (HL). Samples collected from Cross Lake, Lac du Bonnet and Red Lake were pooled into one vast population called Lac du Bonnet (LB). In Quebec, samples collected from either side of the Gatineau River were pooled as Gatineau (GT), the regions of Waskaganish and Eastmain were pooled as Eastmain (EA), and the regions of Godbout and Port Cartier were pooled as Port Cartier (PC) (see Fig.6-1).

H_E values were relatively homogeneous (average H_E=62.6%) among all sampled regions with the exception of the Newfoundland population (H_E=40.2%) that was found significantly lower (Wilcoxon's signed ranks test; Sokal and Rohlf 1995; Table 6-1). H_E in the Yukon and Northwest Territories was slightly higher than most regions, but not significantly.

Pairwise F_{ST} and the genetic distances D_S (Table 6-2) and D_{LR} (data not shown) were calculated for all populations. D_S and D_{LR} values were significantly correlated (two-way Mantel test, $r=0.891$, $p<0.001$), as were both genetic distance measures to F_{ST} (F_{ST}/D_S $r=0.962$, $p<0.001$; F_{ST}/D_{LR} $r=0.905$,

$p<0.001$). A Neighbor-Joining tree of D_S values (Fig. 6-2), illustrates that proximate mainland regions are closely grouped genetically relative to the insular Newfoundland population found distant from all other populations.

Rough approximations of pairwise geographic distances were calculated between all population pairs. These values, excluding pairwise values to the island of Newfoundland, were entered into a two-way Mantel test with D_{LR}, D_S, and F_{ST} to test for isolation by distance. All pairwise measures were significantly ($p<0.001$) correlated to geographic distance ($r=0.325$, 0.258, and 0.343, respectively).

Pairwise D_S/geographic distances for martens were also compared to the same measures for the European pine marten (*M. martes* -Kyle et al. submitted, Fig. 6-3). A similar plot, including data from previous studies of fishers (*M. pennanti* - Kyle et al. 2001), wolverines (*Gulo gulo* - Kyle and Strobeck 2001), and brown bears (*Ursus arctos* - Paetkau et al. 1998) is shown in Fig. 6-4. These comparisons reveal the relative level of genetic structuring in each species per unit geographic distance.

The results from the assignment test (Table 6-3) parallel those from the pairwise genetic distances and F_{ST}. Genetic structuring is observed between most populations, as approximately 50%-70% of individuals assign to the region from which they were sampled. However, some regions had as few as 20 % of individuals assigned to the population from which they were sampled, suggesting a lack of genetic structure in these regions. In contrast, in the Newfoundland population, 100 % of individuals sampled in Newfoundland were assigned to Newfoundland.

Discussion

In general, marten populations displayed less genetic structure than we expected. Despite their potential association with old-growth coniferous forests, the suggestion that tracts of treeless land may act as complete barriers to dispersal, and historical reductions in population size in some regions, only weak isolation by distance was observed across the entire sampling range (Fig. 6-2). The exception was the insular Newfoundland population that was found highly divergent from mainland populations. Furthermore, we found little evidence to suggest that marten populations are more genetically structured in the Canadian provinces than in the Canadian Territories (Kyle et al. 2000). Relative to other carnivores, martens are less genetically structured than brown bears (*Ursus arctos* – Paetkau et al 1998)), wolves (*Canis lupus* – Roy et al. 1994, Carmichael et al. 2001), fishers (*Martes pennanti* – Kyle et al. 2001), and European pine martens (*M. martes* – Kyle et al. submitted), but show similar structure to wolverines (*Gulo gulo* – Kyle and Strobeck 2001) and lynx (*Lynx canadensis* – Schwartz et al. 2002).

American pine marten populations, with an average H_E of 62.6%, have a level of genetic variation similar to that of other North American carnivores studied using microsatellites. Fishers, wolverines, brown bears and wolves were found to have H_E values of 62% (Kyle et al. 2001), 63% (Kyle and

Strobeck 2001), 68% (Paetkau et al. 1998), and 63% (Roy et al. 1994), respectively. The values for American pine martens were higher, however, than for their European counterpart, *M. martes*, where an H_E value of 53% was observed (Kyle et al. submitted).

It is unclear how the high level of genetic connectivity observed in this study is maintained between mainland marten populations. If martens do indeed have a limited potential for dispersal in a landscape fragmented by treeless land and roads (Hawley and Newby 1957; Gibilisco 1994; Alexander and Waters 2000; Robitaille and Aubry 2000), there would be a presumed absence of gene flow. We would then suggest that high effective population sizes have slowed the rate of genetic drift, such that little genetic structure is observed between populations, and not enough time has elapsed for the effects of limited gene flow between regions to generate detectable contrasts in genetic structure.

The presumed habitat specificity of martens has been challenged, however (Bowman and Robitaille 1997; Potvin et al. 2000), and it has also been suggested that martens may be insensitive to habitat alterations caused by forestry at a large scale (Mowat 2002). Hence, the low levels of observed genetic structure could be caused by high rates of short-distance migration across a relatively continuous landscape (e.g. Columbian ground squirrel, Dobson 1994). There is evidence that martens are sometimes capable of long-distance dispersal. Telemetry data has shown that martens normally disperse up to 40 - 80km, and it is suspected that more distant movements are possible (Thompson and Colgan 1987; 160 km movement documented in 1999 near northern Ontario-Manitoba border, J.F. Robitaille, pers. comm.).

The lack of genetic differentiation found among mainland populations in this microsatellite-based study parallel the results found in a similar study by Kyle et al. (2000) for northern marten populations, but differ from those based on different molecular markers. Hicks and Carr (1991, 1997), using the mtDNA cytochrome b gene, and McGowan et al. (1999) using RAPD's, found evidence for east-west clades of martens separated by the Rocky Mountains. In contrast, we found little genetic structuring between Alberta and British Columbia populations (Fig. 6-2; Tables 6-2 and 6-3). The discrepancies between the studies using mtDNA and microsatellites reflect the relative rates of mutation in each of the various markers, and the maternal inheritance of mtDNA. Furthermore, male martens are more likely to disperse than females (Buskirk and Ruggiero 1994), and therefore more genetic structure is expected using maternally inherited mtDNA. We note, however, that we did not sample further west than the Slocan and Golden populations in British Columbia (Fig. 6-1) and it is therefore possible that if east-west clades exist these populations could either belong to the eastern clade described in previous studies, or recent gene flow has homogenized populations on either side of the Rocky Mountains.

American pine marten population genetic structure contrasts with observations for European pine martens (Kyle et al. submitted). Relative to European pine martens, American martens exist in an environment that has been less modified, and over a shorter period of time. The persecution of martens in Europe took place for a longer time (Webster 2001), leading to severe population bottlenecks in the early 1900's (Messenger and Birks 2000). These factors may explain why more genetic structure is observed in European pine martens (Kyle et al. submitted) relative to American martens.

Population genetic studies of fishers have generally revealed a high level of structure between populations over relatively short geographic distances (Williams et al. 1999, 2000; Kyle et al. 2001). The level of structure in fishers was attributed to several potential factors, including: habitat specificity, a high degree of philopatry, and historical demographic trends. Both martens and fishers are considered habitat specialists, often existing sympatrically in the Canadian provinces (Ruggiero 1991, Buskirk and Ruggiero 1994), therefore a similar level of genetic structure might be expected in martens. The fact that much more structure was observed among fisher populations may suggest that fishers are more habitat specific and perhaps more philopatric than American pine martens. The severe decline in numbers of fishers during the first half of the 1900's (Powell and Zielinski 1994) may also explain the differing levels of structure observed.

The lack of structure among most wolverine populations was attributed to their impressive dispersal abilities (Kyle and Strobeck 2001) as documented by several authors (Magoun 1985, Gardner 1985, Copeland 1996). The lack of structure among marten populations may be partially explained by dispersal, but martens also occur at much higher densities (see Buskirk and Ruggiero 1994) than wolverines (see Banci 1994). As such, large effective population sizes may slow or delay genetic drift in marten populations.

Newfoundland pine martens (*M. a. atrata*) are listed as endangered by COSEWIC (2001) and have been protected since 1934, yet the population continues to decline. Census size estimates decreased from 630-875 animals in 1986 (Snyder 1986) to only 300 animals in 1995 (Forsey et al. 1995). The low numbers of individuals on Newfoundland have prompted concern that inbreeding might become a problem in this population. Indeed, the level of genetic variation on Newfoundland is significantly less than observed for mainland populations (H_E=40.2% vs 62.6% averaged across all Canadian populations sampled). In this study, we found insular Newfoundland martens to be highly divergent from mainland populations (Tables 6-2 and 6-3; Fig. 6-2).

Our results contrast to those found by McGowan et al. (1999) using RAPDs where Newfoundland martens were found to be most closely related to Labrador martens. Populations on the lower north shore of the St. Lawrence river in Quebec would be assumed to be very genetically similar to those animals found in Labrador. We suggest that our results are concordant with the biogeographic isolation of Newfoundland as found for other Newfoundland mammals (e.g. black bears, Paetkau and Strobeck 1994). The island of Newfoundland has been isolated from the mainland since the last ice age, and movements of mammals between the mainland and Newfoundland are rare (e.g. coyotes, Lariviere and Crete 1993), and undocumented for martens. Without immigration, genetic drift has likely acted to decrease the level of genetic variation on the island, compounded by recent and significant population declines in the marten population on the island. These results are also similar to those found for European pine martens on the islands of Ireland and Scotland where lower H_E values (34% and 42% respectively) were observed relative to mainland populations (Kyle et al. submitted). This insular effect has also documented in Kodiak island brown bears (Paetkau et al. 1998) and Banks island wolves (Carmichael et al. 2001).

It is unclear if the lack of genetic differentiation observed in this study is reflective of the level of genetic structure in other North American marten populations. We suspect that some isolated regions would have more structured populations. Such regions would include many offshore islands where martens exist in British Columbia, Cape Breton, and Prince Edward Island (Gibilisco 1994, Hall 1981), as well as populations within the remnant montane boreal forests of the southern Rocky Mountains (Buskirk and Ruggiero 1994).

Overall, our results revealed no strong breaks in gene flow between mainland regions, but this should be read with caution, as we cannot discard the possibility that smaller-scale habitat disturbances act as partial barriers to marten gene flow (Hargis et al. 1999, Potvin et al. 2000, Forsey and Baggs 2001, Payer and Harrison 2000, Alexander and Waters 2000, Robitaille and Aubry 2000). At a larger scale, however, marten dispersal may not be limited by factors such as road density and deforestation (Mowat 2002), potentially explaining the lack of genetic structure observed.

Acknowledgements

I would like to offer our thanks to those who generously provided us with samples for this study: Garth Mowat and Kim Poole (Aurora Wildlife Research); Rich Weir (Artemis Wildlife Consultants); Fraser Corbould (Peace/Williston Fish & Wildlife Compensation Program); Wayne and Marlene Sharpe (North American Fur Auctions); Fred Neumann (Alberta Trapper's Association); Bruce Trichel and Bob McClymont (Alberta Fish and Wildlife); Barry Verbiwski and Dean Berezanski (Manitoba Wildlife Branch); Jean-Francois Robitaille (Laurentian University); Neil Dawson (Ontario Ministry of Natural Resources), Nathalie Bourbonnais, Michel Hénault, Danielle St. Pierre (Société de la faune et des parcs du Québec); Joe Brazil, Lem Mayo, and Brian Hearn for the Newfoundland samples; and Brian Slough and Philip Merchant (Yukon Renewable Resources).

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Table 6-1. Genetic variation of sampled Canadian marten populations using 11 microsatellite loci (N=sample size, A=average number of alleles, H_E=average expected per cent heterozygosity, Prob. Of ID 1/=probability of identity). Averages are simple average of averages. Population abbreviations given (Abb.).

Population	Abb.	N	A	H_E	Prob. of Id 1/
Newfoundland	NF	46	2.55	40.2	1.90E+04
Eastmain QC	EA	48	6.18	61.9	7.73E+08
Mingan, QC	MI	40	6.27	62.3	2.18E+09
Port Cartier, QC	PC	80	6.64	61.9	1.81E+09
Forestville, QC	FO	40	5.55	59.0	1.51E+08
Gatineau QC	GT	149	6.36	62.3	2.37E+09
Pembroke, ON	PM	30	5.09	56.7	6.57E+07
Chapleau, ON	CH	50	5.82	59.0	4.34E+08
Lac du Bonnet, ON/MB	LB	106	5.97	63.6	4.03E+09
Fort McMurray, AB	FM	23	4.82	61.4	1.85E+08
Whitecourt, AB	WI	30	5.82	65.8	4.45E+09
Edson, AB	ED	17	5.45	68.0	4.35E+09
Nordegg, AB	NO	28	5.55	67.6	3.47E+09
High Level, AB	HL	33	5.64	64.7	1.31E+09
Prince George, BC	PG	25	5.82	64.4	9.48E+08
Chetwynd, BC	CW	44	5.45	62.5	4.23E+08
Golden, BC	GD	60	5.64	65.3	9.74E+08
Slocan, BC	SL	31	5.55	59.5	8.11E+07
Dawson Region YK	DC	108	6.21	66.8	1.18E+10
Watson L. Region YK	LR	78	6.37	66.7	8.72E+09
Fort Providence, NT	FP	30	5.55	64.3	7.23E+09
Fort Smith, NT	FS	30	6.00	66.2	2.96E+10
Fort Simpson, NT	FN	30	5.91	67.2	1.80E+10
Fort Resolution, NT	FR	30	5.27	63.8	2.85E+09
Fort Good Hope, NT	FG	76	5.51	64.3	4.22E+09
Average/Total		**1262**	**5.64**	**62.6**	**1.10E+11**

Table 6-2. Genetic Distances (Upper Diagonal, pairwise F_{ST} ($*10^{-2}$); Lower Diagonal, Nei's standard genetic distance, D_S ($*10^{-2}$)).

	NF	EA	MI	PC	FO	GT	PM	CH	LB	FM	WI	ED	NO	HL	PG	CW	GD	SL	DC	LR	FP	FS	FN	FR	FG
NF	0	19	22	22	24	21	21	25	19	28	21	25	24	24	26	25	21	25	20	21	22	22	24	30	20
EA	27	0	1.4	2.1	3.7	1.3	2.0	3.0	1.3	6.3	3.2	4.8	3.4	4.2	3.4	5.8	4.2	7.7	5.0	3.9	3.1	3.1	3.7	6.9	4.5
MI	33	4.2	0	1.5	3.0	1.8	3.7	4.3	2.4	6.6	4.3	4.1	3.2	3.9	3.9	6.3	4.9	7.7	4.4	3.3	3.2	3.0	3.0	6.9	4.2
PC	35	4.9	4.2	0	2.8	1.9	4.0	4.5	3.0	7.5	5.3	5.4	4.1	5.3	4.7	6.2	4.6	7.9	5.8	4.5	4.2	3.6	3.7	5.7	4.7
FO	35	7.9	6.9	6.0	0	3.9	4.2	6.1	3.9	8.6	7.0	6.3	5.2	7.0	6.2	6.8	6.4	11	6.9	5.5	5.4	5.5	4.7	7.8	6.3
GT	35	3.4	4.5	4.1	7.7	0	3.1	2.8	1.7	6.8	4.0	5.5	3.4	4.3	4.4	6.3	5.5	8.8	5.3	4.1	3.5	3.1	3.8	5.9	4.4
PM	27	4.9	7.9	7.9	8.2	6.3	0	5.3	4.0	12	6.9	8.9	7.4	7.9	8.0	9.1	7.4	12	8.6	7.6	6.2	5.1	7.0	11	7.1
CH	38	6.4	8.8	8.8	11	5.7	10	0	2.8	8.5	5.1	7.6	4.8	5.7	5.0	6.8	6.3	11	7.0	5.1	3.9	3.8	5.5	7.2	5.0
LB	32	3.6	5.9	6.3	8.0	3.7	8.1	5.9	0	6.3	3.1	3.6	2.2	3.6	3.6	5.2	3.5	7.5	4.6	3.5	2.5	2.9	3.5	6.2	4.3
FM	46	14	15	16	18	15	24	17	15	0	5.7	3.7	4.0	6.5	2.3	5.0	4.7	7.5	3.7	3.7	5.5	6.3	2.6	5.9	4.7
WI	32	8.2	11	12	15	9.3	14	11	8.2	14	0	2.2	1.7	0.7	2.2	5.6	2.1	5.2	1.5	1.8	1.4	2.5	1.7	5.3	1.7
ED	41	13	12	14	15	14	19	18	11	11	9.0	0	1.1	3.5	2.0	6.2	2.6	5.4	2.0	1.7	2.9	4.1	1.4	5.1	3.1
NO	41	9.1	9.1	10	12	8.5	16	11	6.7	11	7.0	7.3	0	1.9	1.4	3.7	1.3	4.1	2.2	1.6	2.0	2.0	1.3	4.4	2.7
HL	40	10	9.8	12	15	9.7	16	12	8.9	16	4.4	12	7.2	0	3.2	5.9	3.0	7.2	2.5	1.9	1.2	2.0	1.4	6.3	2.6
PG	42	8.9	10	11	14	13	17	11	9.4	7.6	7.7	8.9	6.7	9.7	0	2.3	2.2	3.9	1.4	1.1	1.8	2.8	1.8	4.0	1.7
CW	41	12	13	13	14	13	18	14	12	11	13	16	10	14	6.8	0	3.3	5.1	3.3	2.9	3.3	5.2	3.8	5.4	2.7
GD	33	9.3	11	10	13	11	15	13	8.5	12	6.1	9.2	5.6	7.4	6.6	8.0	0	3.2	2.6	2.3	1.8	3.2	2.3	5.8	2.1
SL	37	16	16	16	21	18	22	22	16	16	12	13	10	16	9.6	11	8.1	0	3.7	4.9	4.8	6.3	5.2	7.3	4.0
DC	36	11	11	13	15	11	18	15	11	9.4	5.1	8.0	7.3	7.2	5.3	8.2	6.6	8.4	0	0.8	1.8	3.1	1.5	4.2	0.9
LR	36	8.8	8.0	9.7	12	8.7	16	11	8.1	9.5	5.9	7.3	5.9	5.9	4.8	7.3	5.7	11	2.9	0	1.5	2.3	1.1	4.7	1.0
FP	34	8.0	8.5	9.6	12	8.3	13	8.7	6.8	14	5.8	10	7.5	5.3	7.0	8.6	5.6	11	5.6	5.1	0	1.5	0.8	3.6	0.9
FS	35	8.1	8.1	8.5	12	7.6	11	8.6	7.8	16	8.2	13	7.8	7.0	9.0	13	8.1	14	8.7	7.2	5.9	0	2.0	5.9	2.8
FN	39	9.5	8.3	9.0	11	9.0	15	12	9.3	8.1	6.8	7.7	6.5	5.9	7.2	9.9	6.5	12	5.5	4.5	4.8	7.4	0	3.0	1.6
FR	53	16	16	13	17	13	22	15	15	14	14	15	13	16	11	13	14	16	11	12	10	15	9.3	0	4.3
FG	32	9.9	9.7	10	13	9.2	14	10	9.5	11	5.5	10	7.9	7.1	5.7	6.9	5.7	8.8	2.8	3.2	3.8	7.8	5.4	11	0

Table 6-3. Assignment Test: Percentage of Assignments (left colum: sampled from...; top row, assigned to...

	NF	EA	MI	PC	FO	GT	PM	CH	LB	FM	WI	ED	NO	HL	PG	CW	GD	SL	DC	LR	FP	FS	FN	FR	FG
NF	**All**																								
EA		**38**	8.3	6.3	4.2	21	4.2	4.2	2.1		2.1			4.2			2.1					4.2			
MI		7.5	**43**	10	10	7.5	2.5		2.5				2.5	2.5	2.5				2.5	5.0		2.5			
PC		11	7.5	**55**	7.5	3.8	2.5	1.3					1.3	1.3		1.3				1.3	2.5	2.5			1.3
FO			7.5	15	**55**	2.5			2.5	5.0							2.5						5.0	2.5	2.5
GT		10	4.0	4.7	5.4	**47**	3.4	5.4	5.4	0.7	0.7	0.7	3.4	0.7		0.7	1.3	0.7	0.7	0.7	0.1	1.3	2.0		0.7
PM		6.7			3.3	10	**63**	6.7	3.3													3.3			3.3
CH		4.0	2.0	4.0	2.0	4.0	4.0	**60**	2.0		2.0				4.0	2.0	2.0			2.0	4.0		2.0		
LB		3.8	2.8	0.9	3.8	9.4	4.7	4.7	**48**	2.8	0.9	2.8	2.8		1.9	1.9	1.9	0.9			3.8		0.9		0.9
FM		4.3			4.3				4.3	**61**			4.3	4.3		4.3	4.3		4.3				4.3		
WI		3.3			3.3			6.7			**37**	3.3		10		6.7			10		3.3		6.7	3.3	6.7
ED			5.9						5.9			**35**	18					5.9		5.9	12				12
NO									7.1	3.6	3.6	7.1	**32**	3.6	11	3.6	3.6	3.6	3.6	7.1	3.6		3.6		3.6
HL		12							3.0		9.1		6.1	**34**	3.0	6.1		3.0	6.1	3.0		3.0	12		
PG			4.0		4.0					12	4.0			4.0	**28**	8.0		4.0			12	4.0	4.0	4.0	8.0
CW			2.3		2.3			2.3	4.5	2.3					14	**61**	2.3		2.3				4.5	2.3	
GD		3.3		5.0	3.3			3.3		1.7		3.3	5.0		1.7	3.3	**45**	10		1.7	3.3			3.3	8.3
SL												3.2			6.5	3.2	16	**65**	3.2						3.2
DC		0.9	0.9	0.9	1.9			1.9	0.9	3.7	10	2.8	1.9	4.6	3.7		1.9	1.9	**41**	5.6	5.6	0.9	3.7	0.9	5.6
LR			1.3		1.3			3.8		5.1	2.6	3.8	5.1	2.6	5.1	1.3	2.6	1.3	9.0	**36**	2.6	5.1	1.3	1.3	9.0
FP		3.3	6.7		3.3	10						3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**20**	3.3	6.7	6.7	10
FS		3.3	3.3	6.7					3.3						3.3	3.3			6.7	3.3	3.3	**60**			3.3
FN			3.3							17	3.3			10	3.3		3.3	3.3	6.7	6.7	3.3		**23**	3.3	13
FR								3.3		3.3		3.3	6.7	3.3		3.3					3.3		3.3	**70**	
FG				1.3		1.3	1.3	2.6		2.6	6.6	1.3		6.6	2.6	1.3	5.3	1.3	12	6.6	5.3		2.6	1.3	**38**

Figure 6-1. Map of sites sampled for American pine martens. See Table 1 for abbreviations. Circles surrounding sampled sites represent regions pooled (based on similarity of genotypic frequencies) for all genetic analyses.

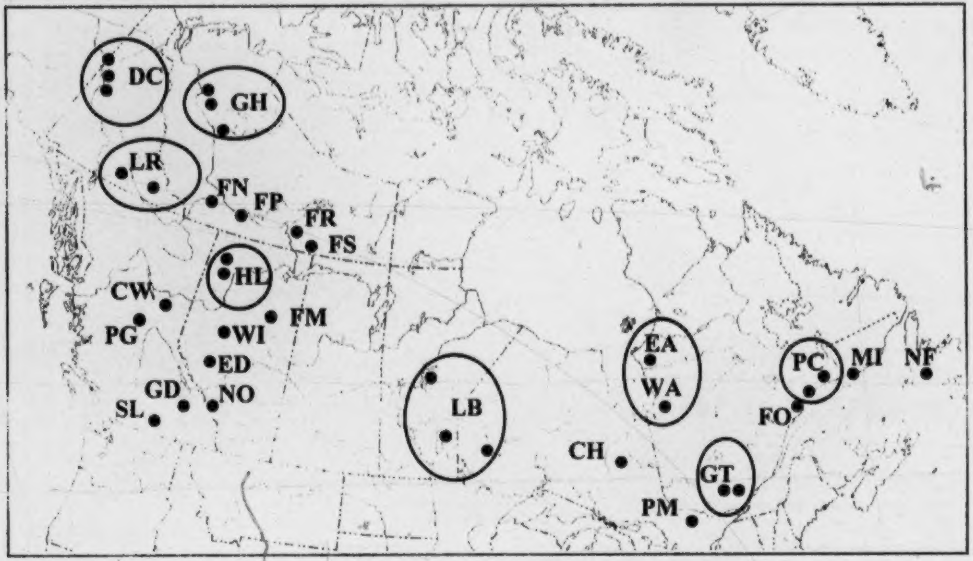


Figure 6-2. Neighbor-Joining Tree of pairwise genetic distances, D_S, between all marten populations. See Table 1 for abbreviations.

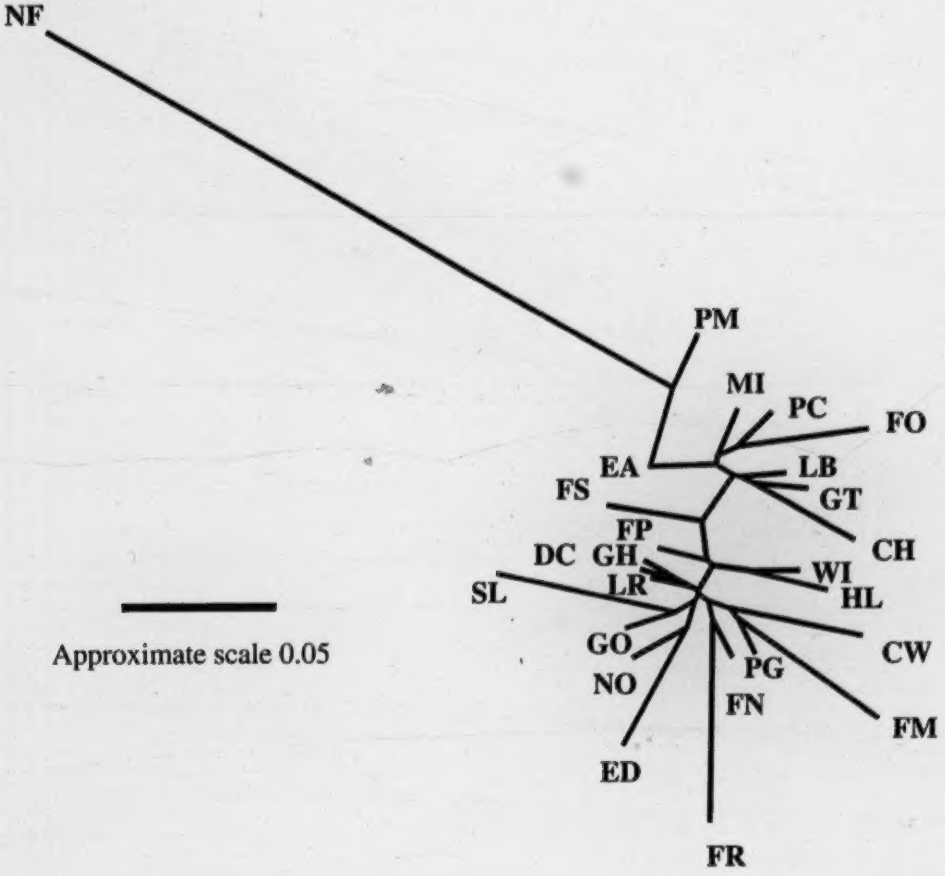


Figure 6-3. Plot of genetic distance (D_S) per unit distance (km) in *M. americana*, excluding Newfoundland martens (Slope=0.0208/1000km, S.E.=0.0018) and European pine martens (mainland populations, slope=0.140/1000km, S.E.=0.018, Kyle et al. submitted).

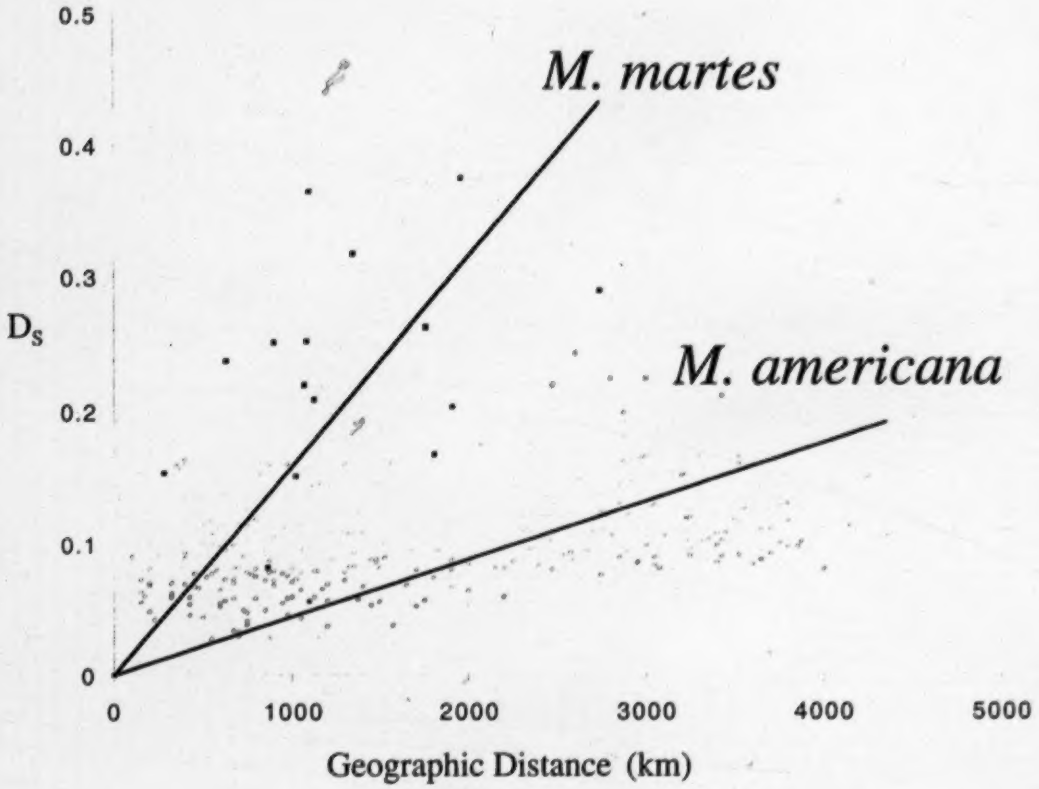
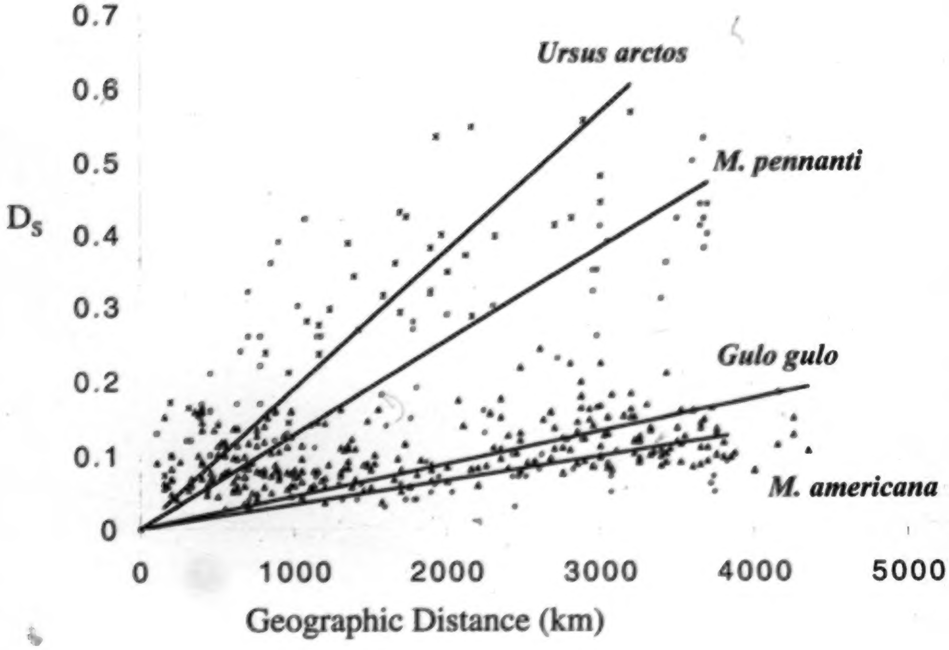


Figure 6-4. Plot of genetic distance (D_S) per unit distance (km) in *M. americana*, excluding Newfoundland martens (Slope=0.0208/1000km, S.E.=0.0018) and other North American carnivores, including: wolverines (Slope=0.0183/1000km, S.E.=0.005, Kyle and Strobeck 2001), fishers (Slope=0.092/1000km, S.E.=0.008, Kyle et al. 2001), and brown bears (Slope=0.137/1000km, S.E.=0.013, Paetkau et al. 1998).



Chapter 7

Genetic structure of European pine martens (*Martes martes*), and further evidence of introgression of *M. americana* in the vulnerable English population*

Introduction

European pine martens (*Martes martes*) are mid-sized mustelids that occur throughout most of western Europe, including Fennoscandia, but excluding parts of the Low Countries. Persecution by dog hunts, poisoning, and trapping, as well as habitat loss and a concomitant increase in predation by foxes, have all contributed to a general decline across much of their distribution (Langley and Yalden 1977; O'Sullivan 1983; Webster 2001). The fragmentation of the species' range may have decreased levels of gene flow among regions and resulted in a loss of genetic variation, potentially limiting the evolutionary potential and increasing the risk of extinction for this species (Caro and Laurenson 1994; Lande and Shannon 1996). However, the patterns of population change are different depending upon the particular local factors that operate. Pine martens are protected in Britain, so that in Scotland the population continues to expand following an early 1900's bottleneck. In contrast, the species remains rare and difficult to monitor in England and Wales, where its status is disputed (Bright et al. 2000; Messenger and Birks 2000).

A previous genetic study of *M. martes* used the control region and a cytochrome *b* fragment of mtDNA to investigate the phylogeography of this species (Davison et al. 2001). A general lack of ancient lineages in martens (and polecats *Mustela putorius*) indicated that the present-day animals in central and northern Europe may have colonized from a single European refugium following a recent glaciation. However, genetic structuring was still present, especially involving comparisons with Ireland, Finland or Scotland. Davison et al. (2001) also reported evidence for historic introgression with the sable (*M. zibellina*) in Fennoscandia, along with mtDNA and morphological evidence for American pine martens (*M. americana caurina*) in England.

Population genetic studies, using nuclear markers, have already been conducted on other *Martes* species. *M. americana* sampled from the Canadian territories had a very low level of genetic structure across vast geographic regions, despite the presence of potential barriers to gene flow, such as the MacKenzie mountain range, as well as an extensive harvest of this species (Kyle et al. 2000).

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The lack of structure was attributed to high levels of gene flow among regions, high effective population sizes, and relatively continuous habitat. In a similar study, fisher populations (*M. pennanti*) sampled from across the Canadian provinces revealed relatively high levels of genetic structuring over short geographic distances (Kyle et al. 2001). The level of structure in this species could be a reflection of philopatry, or potentially, the large demographic changes that took place in most populations of this species in the early 1900's. Fishers were extirpated from much of their range as a result of anthropogenic influences (logging and fur harvests). Consequently, only a discontinuous distribution of small fisher populations remained across most of its range, not unlike the situation for *M. martes*.

Though the life history traits of the European pine marten most closely resemble those of the American pine marten, the level of habitat fragmentation in Europe is expected to be significantly higher than that found in the Canadian north (essentially continuous habitat). For this reason, we might expect a level of structure more similar to that found in *M. pennanti* compared with *M. americana*.

In this study we attempt to obtain a contemporary view of the levels of gene flow among central and northern populations of *M. martes* using microsatellites. These fast evolving markers can potentially reveal barriers to gene flow among regions while not necessarily reflecting genetic patterns strongly influenced by the last ice-age. Further goals of this project were to investigate the levels of genetic variation and structure among European pine marten populations relative to other mustelid species, to determine the origins of individuals from the vulnerable English population, and finally, compare microsatellite data to existing mtDNA data for this species.

Materials and Methods

Sampled Locations

Samples of *M. martes*, in the form of extracted DNA, were obtained from England, Scotland, Ireland, Italy, Germany, Latvia, Netherlands, Sweden, and Finland (see Figure 7-1 for map and Davison et al. 2001 for sample collection details). In northern and central Europe, *M. martes* are broadly sympatric with beech martens (*M. foina*), but the species are easily distinguished. In much of southern Europe, *M. martes* is rare or absent. Further east, the data on species distributions are sketchy (Anderson 1970; Bakeyev and Sinitsyn 1994). The sable, *M. zibellina,* is distinguished from *M. martes* by pelt and skull characters, but it is not present in western Europe (Mitchell-Jones et al. 1999). It replaces *M. martes* at some point east of the Ural mountains, and the two species may hybridize when they meet (Grakov 1994). In England and Wales, marten samples were very difficult to collect, with only eight recent records; seven from England and a Welsh marten scat (Davison et al. 2001, 2002). Sample locations for England are detailed in Table 7-1. For comparative purposes, sixteen *M. americana* samples from the

Yukon and Newfoundland in Canada were run at the same microsatellite loci as the *M. martes* samples.

Amplification and Visualization of DNA

Eleven microsatellite primer sets were used in this study, originally developed by: Davis and Strobeck (1998) in martens (MA-1, MA-2, MA-9, MA-18, and MA-19) and wolverines (GG-7, GG-14); by Dallas and Piertney (1998) in Eurasian otters (L-604); by Flemming et al. (1999) in mink and ermine (Mvis 020); and by Walker et al. (2001) in wolverines (Ggu 452, Ggu454). PCR amplification was performed as in Davis and Strobeck (1998). DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. The programs GeneScan™ Analysis 2.02 and Genotyper® 2.0 were used to analyze the DNA fragments.

Tests of Disequilibrium and Heterogeneity

Departure from Hardy-Weinburg equilibrium (H.W.E.) and genotypic disequilibria were assessed, for each of the loci, using Genepop 3.1 (Raymond and Rousset 1995). Multiple comparisons were accounted for using the Dunn-Sidak experiment-wise error rate. A *G*-test for heterogeneity, summed among loci (Sokal and Rohlf 1997), was then performed for each pair of sampled areas.

Genetic Variation

The relative genetic variation in each population was assessed using allele frequency data; mean number of alleles, unbiased expected heterozygosity (H_E, Nei and Roychoudhury 1974), and unbiased overall probability of identity (P_{ID}, Paetkau et al. 1998) were calculated. Wilcoxon's signed-ranks test was used to test for significant differences in heterozygosity levels among populations (Sokal and Rohlf 1997).

Genetic Distances and pairwise Fst

Genetic distances between populations was estimated using Nei's standard genetic distance, D_S, (Nei 1972) and the genotype likelihood ratio, D_{LR}, (Paetkau et al. 1997). Both D_S and D_{LR} were calculated using programs within the website, www.biology.ualberta.ca/jbrzusto/Doh.php, (designed by John Brzustowski). These two genetic distances, based on the infinite allele model, are more appropriate than distances based on the stepwise mutation model when, as in this study, some imperfect microsatellites are used (Paetkau et al.1997; Forbes and Hogg 1999). Genepop 3.1 was used to calculate pairwise F_{ST} estimates (as per Weir and Cockerham 1984).

Assignment Test

The assignment test (Paetkau et al. 1995), also found on the above web site by John Brzustowski, was run for all populations. This test determines the probability of a genotype occurring in the region from which it was sampled, and the probability of it occurring in each of the other sampled regions. It then assigns each individual to the population in which that individual's genotype has the highest probability of occurring (see Waser and Strobeck 1998). We also ran this test making no assumptions about the heterogeneity of the martens from England, but simply added the genotypes from these individuals into a pairwise comparison of Yukon *M. americana* and Scottish *M. martes* populations, in an attempt to reveal any *M. americana* or *M. americana/M. martes* hybrids among the English samples.

Isolation by Distance

A two-way Mantel test (Mantel 1967), found on Pierre Legendre's webpage: http://www.fas.umontreal.ca/BIOL/legendre/, was used to evaluate the correlation between the genetic and geographic distances. The pairwise geographic and genetic distances of *M. martes* populations were also plotted against *M. americana* pairwise distances (from Kyle et al. 2000) to illustrate the difference among the species. Regressions of each curve were calculated using Excel and were compared with regressions of other mustelid species.

Results

Tests of Disequilibrium and Heterogeneity

Three loci were dropped prior to any analyses for heterogeneity and disequilibria: MA-9 revealed 1bp alleles from 140-144, but many samples did not amplify at this locus, Mvis 020 revealed alleles from 160-188, but again, many samples did not amplify cleanly at this locus, and Ggu 452 was dropped because it had only two alleles.

All sampled regions, genotyped using eight loci, conformed to Hardy-Weinberg equilibrium (H.W.E.), accounting for experiment-wise error, with the exception of Scotland at locus MA-18 and Finland at locus GG-14. Both deviations from H.W.E. were heterozygous deficits, implying that null alleles might exist at these loci in these populations. As these deviations only occurred at one locus in each of these populations, all loci were retained for analyses. One deviation from genotypic equilibrium was revealed with locus MA2 and locus Ggu454 in the Scottish population. As genotypic disequilibrium was only found to exist in one pair of loci in one population, all eight loci were retained for all analyses. G-tests and assignment tests (randomizing combined gene pools; data not shown) both suggest that all regions sampled differed significantly in their genotypic frequencies (α=0.05) and were treated as distinct populations for all subsequent analyses.

Genetic Variation

Levels of genetic variation are summarized in Table 7-2. Both Scotland and Ireland had significantly lower levels of H_E as compared to continental populations (using Wilcoxon's signed-ranks test, α=0.05). All continental populations had relatively homogenous levels of genetic variation, with no significant differences among them. The English population was anomalous, having a relatively high level of genetic diversity compared with Ireland and Scotland. This was probably due to *M. americana* or hybrids in the English population sample (see discussion).

Another comparison was performed using seven microsatellite loci between North American *M. americana* and European *M. martes* populations (locus Ggu454 did not amplify in *M. americana*). The *M. americana* population in the Yukon had the highest level of genetic variation, although not significantly different from continental *M. martes* populations. The isolated *M. americana* population on the island of Newfoundland (*M. americana atrata*, listed as endangered by COSEWIC) had a similar level of genetic variation to Scottish and Irish *M. martes* populations.

Pairwise Genetic Distances and Fst

Pairwise estimates of Nei's standard genetic distance, D_S, were found to be highly correlated with both the likelihood ratio genetic distance, D_{LR} (R=0.91, p=0.00006), and pairwise F_{ST} (R=0.84, p=0.0003). D_S was also significantly correlated with geographic distance between populations (R=0.55, p=0.007). Pairwise F_{ST} did not correlate as strongly with geographic distance as did the genetic distance measures (R=0.31, p=0.11).

Both genetic distance values and pairwise F_{ST} suggest that Scottish and Irish martens are differentiated from the continental distribution of pine martens (see Tables 7-3 and 7-4). Furthermore, these populations are as differentiated from each other as they are from the continental populations. The level of structure observed in the northern continental populations (Sweden, Finland, and the Netherlands) are moderately high as compared to among the more southerly populations of Germany, Italy, and Latvia. In general, the levels of structure are moderate with an overall F_{ST} value of 0.18 (eight loci).

The pairwise D_S values were plotted against geographic distance between populations (Figure 7-2). A linear regression of the data revealed a D_S value of 0.140/1000km (S.E.=0.018) for the continental *M. martes* populations. When the Scotland and Ireland populations were included the value was 0.198/1000km (S.E.=0.05).

Assignment Test

The assignment test results, using eight loci, in the absence of *M. americana,* populations, also support the suggestion that both Scotland and Ireland are genetically differentiated from the continental populations, with 93 and 100% of the individuals assigning to the population from which they were sampled, respectively (see Table 7-5). The next most structured populations were in northern Europe with Finland and Netherlands having over 77 % of individuals assigned to the population from which they were sampled. The more central continental populations all shared more cross-assignments (<40% of assignments to the populations from which they were sampled), including Latvia, Germany, and Italy.

The assignment test was also run including the *M. americana* samples (data not shown). There was little effect on the *M. martes* assignments with or without the *M. americana* samples included. Both *M. americana* populations were found to be completely distinct in this test, with all individuals assigned to the populations from which they were sampled and with no cross-assignments to the *M. americana* populations.

The genotype probabilities from the assignment test were also plotted on a graph using individuals from the Yukon (*M. americana*), Scotland (*M. martes*) and England (Figure 7-3). Three individuals from Northern England had intermediate probabilities of being from Scotland or the Yukon. This raises the possibility that these animals are *M. americana/M. martes* hybrids. However, a significant problem is that we probably sampled only a proportion of the genetic variation in *M. americana*, from a limited geographic range. The two other Northumbrian martens fell in the centre of the Scottish group, whereas the samples from Lancashire and Yorkshire were more distinct, on the outer edge of the Scottish group (Figure 7-3).

Discussion

Martes martes populations had lower levels of genetic variation and higher levels of genetic structure compared with other *Martes* and mustelid species. We suggest these results are most likely due to the relative differences in the level and duration of recent anthropogenic disturbances in Europe and northern North America. The structure uncovered in this study could also reflect a greater degree of philopatry in this species compared with *M. americana*. However, *M. martes* are believed to be a vagile species, consistent with evidence for long distance gene flow (Table 7-5). Another alternative is that more ancient processes still influence the gene frequencies, such as post-glacial founder effects and introgression from *M. zibellina* in Fennoscandia.

The analysis also extends the evidence for the presence of *M. americana* individuals in England, and raises the possibility of hybridization with indigenous *M. martes*. This finding may have a significant bearing on current discussions on the status of English martens and the appropriateness of proposed re-introductions.

Genetic Variation and Structure Relative to other Mustelid Species

M. martes populations had an average H_E of 53%, excluding England, and 58%, further excluding the island populations of Scotland and Ireland. Although it is difficult to make a direct comparison with other studies, since different loci were used, *M. martes* had a lower level of genetic variation than northern Canadian *M. americana*, H_E=66% (Kyle et al. 2000); Canadian *M. pennanti*, H_E=62% (Kyle et al. 2001); and North American wolverines, H_E=63% (Kyle and Strobeck 2001); but higher than Scandinavian wolverines, H_E=37% (Walker et al. 2001).

The pairwise genetic distances and F_{ST} found in *M. martes* were higher than in other mustelid species. Here, a moderately high overall F_{ST} value of 0.18 at eight loci was obtained for *M. martes* relative to that found for: *M. americana*, F_{ST}=0.02, *M. pennanti*, F_{ST}=0.14, North American wolverines, F_{ST}=0.05, and Scandinavian wolverines, F_{ST}=0.05. The results obtained from the assignment test for *M. martes* were more ambiguous. Both Scotland and Ireland were found to be relatively isolated, but for the continental populations, only the Netherlands and Finland had a high percentage of individuals assigned to the population from which they were sampled. Germany, Italy, Latvia, and to a lesser degree Sweden had many cross-assignments to other sampled regions.

To illustrate the differences in genetic structuring among the mustelid studies, a linear regression of the D_S and geographic distances was performed. *M. martes* had the highest level of structure per unit distance, 0.140/1000km (S.E.=0.02) using continental populations alone, followed by *M. pennanti* 0.092/1000km (S.E.=0.008), *M. americana* 0.057/1000km (S.E=0.009), and then North American wolverines 0.018/1000km (S.E.=0.005). The level of genetic structuring observed among *M. martes* populations may be a result of recent population fragmentation and bottlenecks. Historically, the continental populations may have existed as a more panmictic unit, as found with *M. americana* from the Canadian north (Kyle et al. 2000).

Origins of M. martes in England

In England and Wales controversy exists as to the origins and status of pine martens. Despite evidence for populations persisting through the 20th century (Strachan et al. 1996), some authors have suggested that no viable populations remain and reintroduction to England has been proposed (Bright et al. 2000). Others have argued that while any martens remain, we should try to understand their failure to expand (Messenger and Birks 2000). We have eight recent records (seven English martens and one Welsh marten scat;

Davison et al. 2001, 2002), in addition to a sightings survey (Messenger and Birks 2000). Here we used a pairwise comparison of pairwise genotypic probabilities between Scotland and the Yukon to determine the probability of English samples being from either population (Figure 7-3). Three of the seven individuals had genotypes with intermediate probabilities of being from the *M. martes* population in Scotland and the *M. americana* population from the Yukon, Canada. The other four samples from England fell with the Scottish samples, though two were on the edge of this group. The combined results from the mtDNA and nuclear markers suggest that some remaining English animals may be indigenous, while some animals are *M. americana* or hybrid *M. americana/M. martes*.

The most likely source of *M. americana* in the North of England are martens that escaped or were released from commercial mink farms. This raises the possibility of hybridization between species in captivity. Unfortunately, there are no official records for farms in Northumberland prior to the 1962 Mink (keeping) regulations, although they were present from the 1920's. From 1962, the number of farms in Northumberland varied between two and six, though none are present now. Interestingly, with mink, the original stock animals came from the Hudson Bay area, but they were later superseded by a heavier strain from north of the Yukon River and Alaska (Kevin O'Hara, pers. comm.). There were also many 'back garden' farmers prior to 1962, as well as an Arctic Fox farm with stock from Alaska and Canada.

Comparison of mtDNA and microsatellite population structure

A study by Davison et al. (2001) revealed significant mtDNA structuring among the continental populations, possibly due to a low level of maternal gene flow, or arising from the post-glacial colonization of Europe. Similarly, using nuclear markers we have uncovered moderate to high levels of genetic structuring among the continental populations. The study by Davison et al. (2001) also revealed two continental European mtDNA lineages, one found throughout Europe, with another found only in Finland and Sweden. It is likely that the latter lineage arose by introgression from *M. zibellina*. The microsatellite results may provide some support for a distinct Fennoscandian group, with elevated genetic distance values between Fennoscandia (especially Finland) and the central European populations.

Units for conservation

Many of the population samples were significantly differentiated by mitochondrial type (Davison et al. 2001), microsatellite genetic variation and population structure, and could be considered separate Management Units (*sensu* Moritz 1994). However, the greater explanation for the genetic differentiation may be recent population fragmentation and bottlenecks. If martens were once more continuously distributed, then some of the differences in allele frequencies could be alleviated. For a similar circumstance in wolves, Vila et al. (1999) suggest that individuals from neighbouring or closely related populations can justifiably be used as a source for re-introduction or population augmentation. Although *M. martes* haplotype frequencies have probably been less affected by population fragmentation and bottlenecks compared with wolves, we believe such a system could be applied to this species. However, both the precautionary principle and common sense suggest that animals should not be translocated unless absolutely necessary, especially to island populations.

Acknowledgements

Special thanks to all persons who collected samples, especially from England and Wales, to Kevin O'Hara (Northumberland Wildlife Trust) for researching mink farms, and to The Vincent Wildlife Trust for developing our interest in *Martes martes*. I would also like to thank Joe Brazil, Lem Mayo, and Brian Hearn for the Newfoundland samples and Philip Merchant for the Yukon samples.

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Table 7-1 Sources and mtDNA haplotype (Davison et al. 2001) of all known recent English martens. Haplotypes w and x are from *M. americana*. Vincent Wildlife Trust identification number (VWT ID).

English County	**Year**	**mtDNA haplotype**	**Source**	**VWT ID**	**Reference**
Lancashire	1994	a	Ellen Davies	139/ Mama5	Birks et. al. (1997)
Yorkshire	1993	a	Charles Critchley	176/ Mama155	Jeffries and Critchley (1994)
Northumberland	1994	a	Colin Simms	99/ Mama15	
Northumberland	1995	a	Colin Simms	397/ Mama6B	
Northumberland	1990's	w	Colin Simms	Mama6A	
Northumberland	1990	x	Colin Simms	473/ Mama16	
Cumbria	1995	x *	Colin Simms	523/ Mama117	

* not reported in Davison et al. (2001)

Table 7-2 Genetic Variation (at 7 loci)

Population	Abbrev.	N	#alleles	H_E (%)	P_{ID} 1/
Scotland	Sco	59	3.86	42.27	955
England	Eng	7	3.57	66.06	76,300
Ireland	Ire	9	1.86	33.99	156
Germany	Ger	10	3.86	56.24	27,400
Sweden	Swe	16	3.86	57.28	26,500
Finland	Fin	26	4.57	57.15	15,100
Netherland	Net	10	3.57	53.76	8.690
Latvia	Lat	8	3.86	63.78	58,500
Italy	Ita	15	4.57	61.04	53,500
Newfoundland	N F	16	2.43	44.55	554
Yukon	YK	16	3.86	68.96	1,540,000

Table 7-3 Genetic Distances (7 loci, with *M. americana*), upper diagonal, D_{LR}, lower diagonal, D_S. Note D_S and D_{LR} correlated (0.91, p=0.00006) by 2-way Mantel test.

	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita	Ne	Yuk
Scot	0	3.34	5.31	4.80	3.44	8.75	7.34	6.91	5.33	27.3	20.3
Eng	.137	0	5.02	2.64	2.73	5.43	5.42	4.59	3.97	20.9	12.9
Ire	.231	.255	0	5.95	5.46	8.62	8.12	9.14	7.43	25.3	17.3
Ger	.230	.195	.327	0	0.68	3.10	1.93	1.85	2.21	21.8	14.9
Swe	.193	.147	.263	.082	0	2.34	3.98	2.97	2.06	22.4	15.5
Fin	.625	.376	.601	.262	.251	0	4.26	1.35	2.01	21.5	13.8
Net	.346	.320	.396	.154	.252	.375	0	3.30	3.70	23.1	14.7
Lat	.473	.304	.591	.219	.237	.151	.317	0	0.41	18.2	12.2
Ita	.484	.362	.347	.208	.203	.289	.365	.167	0	17.1	12.5
Ne	2.22	2.58	2.64	2.21	2.08	1.88	2.52	1.45	1.68	0	9.31
Yuk	1.08	.887	1.13	.895	.910	.664	.765	.650	.904	.727	0

Table 7-4 Pairwise Fst (8loci)

	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita
Scot	0								
Eng	.136	0							
Ire	.223	.199	0						
Ger	.207	.059	.232	0					
Swe	.155	.054	.186	.018	0				
Fin	.316	.165	.324	.129	.120	0			
Net	.242	.118	.257	.061	.112	.187	0		
Lat	.280	.098	.330	.072	.079	.050	.135	0	
Ita	.215	.082	.263	.044	.044	.086	.116	.016	0

Table 7-5 Assignment Test (8 loci, without *M. americana* populations). Left column represents where samples taken from and top row idicates where individuals assigned to.

	N	Sco	Eng	Ire	Ger	Swe	Fin	Net	Lat	Ita
Scot	59	55	0	0	0	3*	1*	0	0	0
Eng	7	3*	3	0	0	0	0	0	0	1
Ire	9	0	0	9	0	0	0	0	0	0
Ger	10	0	0	0	4	3	0	1	2	0
Swe	16	0	0	1	3	9	1	0	1	1
Fin	26	0	0	0	0	2	20	0	4*	0
Net	10	0	0	1*	1	0	0	8	0	0
Lat	8	0	0	0	1	1	2	0	3	1
Ita	15	0	0	0	2	1	2	0	5*	5

*significant at alpha 0.01 by randomization of individual gene pools, assuming HWE.

Figure 7-1. Map of sampled regions.

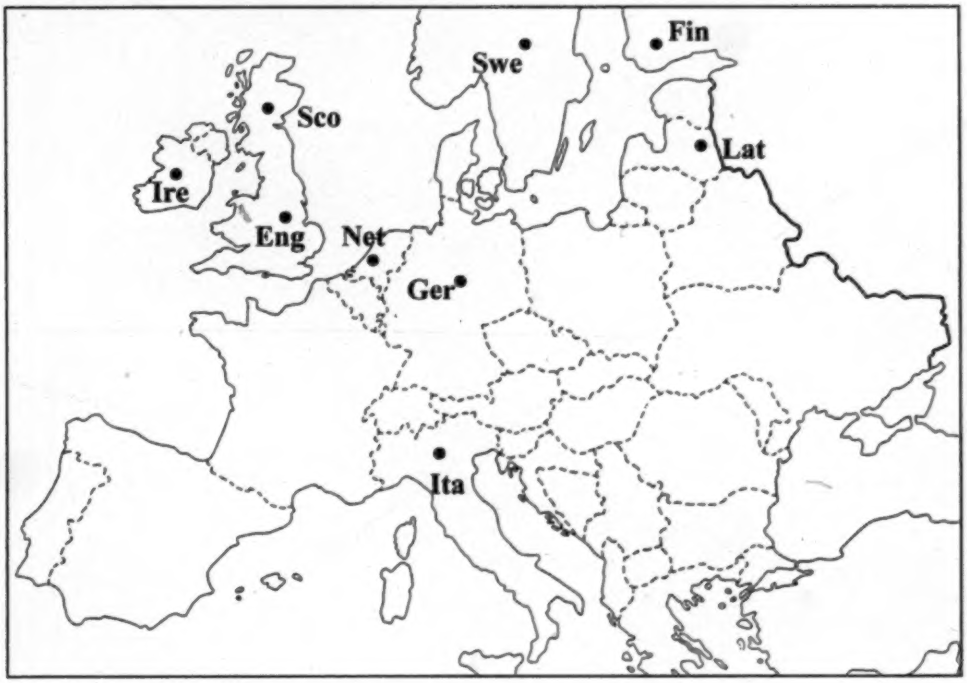


Figure 7-2. Genotype assignments between North American,European martens and potential hybrids from England. Squares are European martens, circles are American martens, and triangles are individuals captured in England.

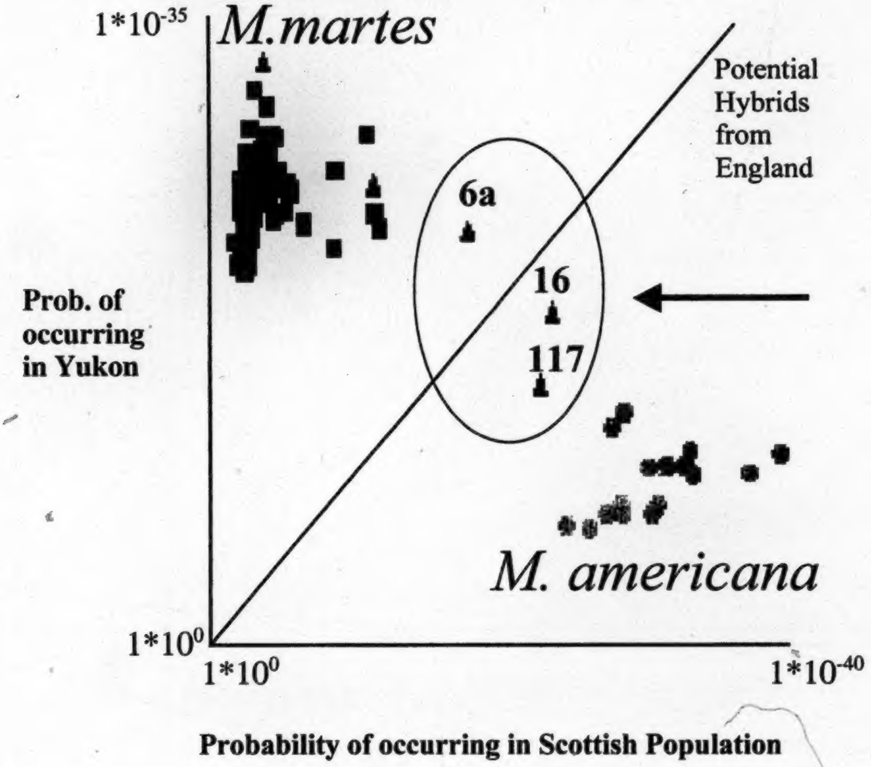
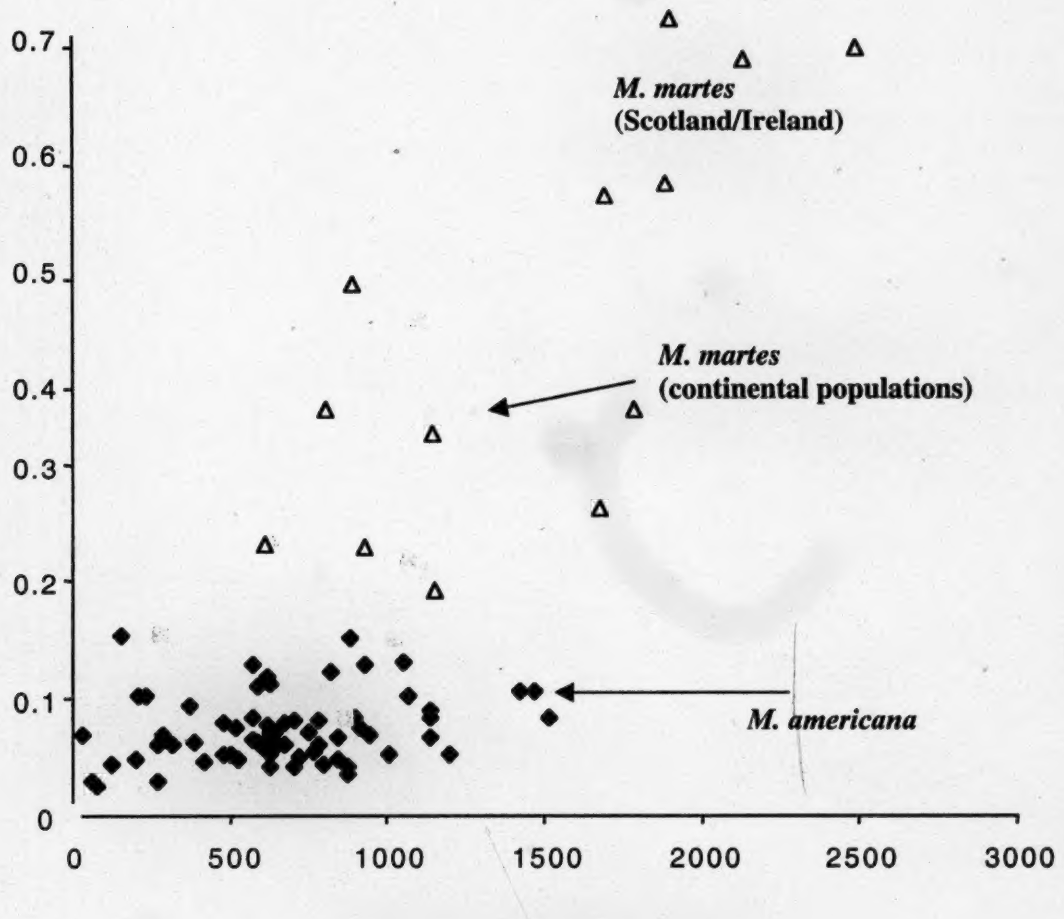


Figure 7-3. Relationship of geographic to genetic distance for both European and American pine martens. Triangles are island populations, squares are continental populations, and diamonds are American pine martens sampled from across Canada. Y-axis is genetic distance, D_S.



Geographic Distance (km)

Chapter 8

Summary

This thesis has provided insight into the genetic variation and structure of three North American mustelids, and one European species, across much of their respective current distributions. Initial predictions that increasing genetic structure would be observed with increasing habitat specificity and decreasing dispersal potential held true to varying degrees in each species. Fishers and European martens, with limited dispersal abilities and strong associations with particular habitat types, were strongly structured. However, American martens with similar characteristics were not strongly structured. Wolverines with the low levels of habitat specificity and high vagility were not strongly structured in northern regions, as expected. These results suggest that those factors influencing genetic structure are multi-faceted, and that life history characteristics other than potential for dispersal and habitat specificity, influence the genetic structure of these species. Furthermore, historical anthropogenic factors, such as trapping and predator control programs, appear responsible for the contemporary trends of genetic structure observed in southern and eastern populations of wolverines, fishers, and European pine martens.

Wolverines

Wolverines were predicted to have little genetic structure given their lack of habitat specificity and their propensity to disperse vast distances. It was also hypothesized that any remnant peripheral populations would be genetically structured as found for Yellowstone brown bears (Paetkau et al. 1998). Northern North American wolverines were found to have very little genetic structure and peripheral regions were strongly structured, as predicted (Chapters 2 and 3). The discrepancies in the level of genetic structure between populations can be partially explained by the varying exposure of these populations to historical anthropogenic influences. Wolverines were largely extirpated from the lower 48 states in the early 1900's (Davis 1939, Newby and Wright 1955, Newby and McDougal 1963) and much of eastern Canada (Banci 1994). Hence, these populations may be remnant populations that have gone through severe population bottlenecks or they have arisen from relatively recent founder events. Either of these factors would have resulted in populations having lower effective population sizes that have become distinct through drift. In northern regions, however, the level of dispersal between regions seems to have maintained relatively homogenous genotypic frequencies over a vast geographic range.

Fishers

Fishers were expected to be moderately structured given their habitat specificity, moderate dispersal abilities, and historical population declines throughout much of their distribution. Fishers were strongly structured across much of Canada (Chapter 4). The structure observed in this species could be the result of several factors, however. Fishers were largely extirpated from most of North America (see Powell and Zielinksi 1994), and as such this species went through population bottlenecks in the early 1900's. These factors likely led to smaller effective population sizes, genetic drift, and finally, structured populations. An alternative hypothesis is that these animals are very habitat specific and are highly philopatric. The habitats that fishers prefer (see Powell and Zielinski 1994) have largely been fragmented across southern Canada, and this may explain the level of structure in this species. In fragmented populations, again, effective population size leading to drift could explain our results.

American Pine Martens

The level of genetic structure between American marten populations was much lower than expected throughout mainland Canada (Chapters 5 and 6). These data might imply that martens are not as habitat specific as presumed (Buskirk and Ruggiero 1994) and that this species may disperse through a landscape in Canada that is largely un-fragmented by road density or deforestation at a large scale (see Mowat 2002). Alternatively, martens populations may be fragmented throughout the Canadian provinces by a lack of old-growth forests, road density, and clear-cutting forestry practices, these results may reflect high effective population sizes in marten populations, such that the effects of genetic drift are not pronounced, despite the fragmentation of suitable habitat.

European pine martens

A high degree of structure was observed in this species in northwestern Europe (Chapter 7). Martens were drastically reduced in numbers and much of their habitat was eliminated through anthropogenic influences over the past few hundred years. Hence, these populations have likely become smaller and more fragmented than North American populations, over time. This has led to smaller effective population sizes, and genetic drift has genetically structured these populations. These data provide a contrast to those findings for North American martens (Chapters 5 and 6), suggesting that Canadian populations may, indeed, become strongly structured over time if exposed to persistent anthropogenic pressures.

Trends Among Mustelids

Initially, martens and fishers were expected to have the highest levels of genetic structure given their life history characteristics of high habitat specificity and low dispersal potential and wolverines would have the lowest levels of genetic structure given their lack of habitat specificity and high vagility. With the exception of peripheral populations, wolverines were found to have the lowest levels of structure, and fishers were found to have the most structure. Martens, however, were found to found to have a similar level of genetic structure to wolverines, despite contrasting life history traits (see Fig. 8-1). The findings for martens were unexpected, and alternatives to limited dispersal potential and habitat specificity (if martens are habitat specific) are needed to explain the observed data. It may be suggested that the relatively high density of this species has had the effect of maintaining genetic homogeneity among regions despite its potentially limiting dispersal capabilities.

All three species are trapped in Canada, but wolverines and fishers have lower densities than martens (see Chapter 1), and as such may be more susceptible to the effects trapping and persecution than marten populations. Furthermore, historical anthropogenetic pressures led to more pronounced declines for wolverine and fisher populations in the Canadian provinces than for martens (with the exception of the maritime provinces; see Chapter 1). Where any of these mustelid populations have been reduced severely in number, they have become structured. This trend also persists in European pine martens, where lower effective population sizes and low densities have likely resulted from anthropogenic pressures that have been present for centuries, and the populations have become strongly structured.

Based on these trends, continued reductions in numbers of these species would result in more structured populations in the future, and such populations are more subject to local extirpation than larger populations (Hanski 1999). A note of optimism comes from the fact that in several regions, mustelids seem to be increasing in number and distribution since the major declines in North America in the early 1900's.

The genetic structuring of these mustelids in North America did not fit any identifiable geographic trends, given the populations we studied. Where wolverines were strongly structured in southern British Columbia, martens were not, while fishers were genetically structured in all regions. Hence, topographic limitations to dispersal are unlikely. Furthermore, where severe population declines have not taken place, as in northern wolverines and most Canadian marten populations, near panmixia seems to exist. It may be suggested that prior to European settlement of North America these terrestrial mustelids would have been largely panmictic, with the exception of insular island populations, and that only weak by isolation by distance would exist among regions.

Trends Among Carnivores

When compared to other carnivores, several interesting similarities and contrasts arise (see Figures 8-1 and 8-2). Wolverines and martens were found to have less genetic structure per unit geographic distance than both brown bears

(Paetkau et al. 1998) and wolves (Roy et al. 1994, Carmichael et al. 2002), but have a similar amount of structure to coyotes (Roy et al. 1994) and lynx (Schwartz 2002). Fishers were also less structured than wolves and brown bears, but more so than coyotes and lynx.

Wolverines and martens, sampled from a similar range to brown bears had much less genetic structure per unit geographic distance. In fact, brown bears were found to be more structured than fishers and European pine martens too. This result is unexpected given the respective potential dispersal ability of brown bears, and may suggest that this species is much more philopatric than other carnivores.

Martens and wolverines displayed a similar amount of structure to lynx. Lynx populations were found to be relatively homogenous across vast geographic regions (Schwartz et al. 2002). The similarity between wolverines and lynx are likely a result of their elevated dispersal abilities. The similarity in structure between martens and lynx is not likely due to similar dispersal abilities. However, lynx, like martens, can be found at high densities in some regions of Canada. Thus large effective population sizes in both species may partially explain the lack of genetic structuring in Canada.

Coyotes did not reveal much genetic structure across their North American distribution (Roy et al. 1994). This result was partially attributed a recent postglacial expansion of the species and relatively large effective populations sizes. These findings can be compared to those for martens where large effective population sizes may have led to little genetic structure. A recent expansion of martens may also be responsible for the trends observed, however microsatellites evolve very quickly and we would only expect to see more recent genetic trends and not remnants of postglacial structure.

Future Directions

This thesis constitutes only the first step in the investigation of the mustelid ecology by genetic means. This work has taken place on a very broad scale, and as such much smaller scaled genetic projects will be able to elucidate yet more of the ecology of these species. Furthermore, this work is likely only reflective of recent historical events influencing the structure of these species. Continued work will be needed to assess the changes in genetic structure of these species from more recent habitat alterations.

Important future studies would include intensive sampling in regions to investigate parentage, mating systems, and social structure in these species; aspects of mustelid ecology that are not well understood at this point. Furthermore, very fine scale studies may also be able to clearly identify those factors influencing the real-time dispersal of individual animals. Studies of this sort are now taking place for brown bears in British Columbia (Proctor et al. in prep), and it would be appropriate to expand on their work using other carnivore species.

Future work may also include remote census', similar to those for brown bears, of wolverines in Ontario, Alberta's park system and adjacent wilderness areas, and southern British Columbia. Such programs have been proposed for several regions (Idaho, Montana, Alberta, British Columbia, and Ontario). It is in these regions where high levels of genetic structure were observed that it is important to determine if these trends of population fragmentation persist at a fine scale and are not just reflective of historical events from population declines in the last century.

Continued finer scale fisher and marten studies would also be appropriate. Results suggest that fishers are potentially more of a habitat specialist than martens, and as such may be the species most influenced by habitat modification. Further study is needed to clarify those factors influencing the lack of gene flow between populations of this species. Only Canadian populations of martens have been investigated in this thesis. Future work on populations of martens that are known to have been naturally isolated in the lower 48 states would be appropriate.

For all of these species, obtaining historical specimens would help identify pre-European settlement dispersal patterns and Ancient samples could reveal post-glacial dispersal patterns of ancient populations (e.g. brown bears, Leonard et al. 2000).

Conclusions

This thesis has attempted to fill a large gap in the literature with respect to mustelid conservation genetics by providing studies similar to those that have taken place for other North American carnivores of concern (see Table 8-1). The few previous mustelid studies that had taken place were limited by several factors including sample size, number of regions sampled, and the lack of resolution provided by other molecular markers. While, as mentioned, this work provides only a preliminary assessment of mustelid population genetics, it does provide further insight into the ecology and future viability of several mustelid species.

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Table 8-1. North American Mid-Large Terrestrial Carnivore Population Genetic Studies (all population genetic studies of mustelids, microsatellite studies of other North American carnivore families). Several species have had no microsatellite work performed on them and this is represented by – in the table.

Species	**Region (s)**	**Markers**	**Results**	**Authors**
Mustelidae				
Gulo gulo	Canadian range, Idaho, Wyoming, eastern Russia	Microsatellites	Nearly panmictic with structure in peripheral regions.	Kyle and Strobeck 2001, 2002.
Gulo gulo	NWT	MtDNA and allozymes	Some structure	Wilson et al. 2000.
Martes americana	Canadian range including Nfld.	Microsatellites	Nearly panmictic, Nfld distinct	Kyle and Strobeck (submitted), Kyle et al. 2000.
Martes americana	Canadian range	RAPDs	Structure	McGowan et al. 1999.
Martes americana	Canadian range	MtDNA	Few haplotypes	Carr and Hicks 1991, 1997.
Martes pennanti	Canadian range, New York (US).	Microsatellites	Strongly structured	Kyle et al. 2001.
Martes pennanti	American northeast	Allozymes	Structured	Williams et al. 1999, 2000.
Mustela vison	Captive and eastern populations	microsatellites	Genetic variation assessment	Belliveau et al. 1999
Mustela nigripes	Remnant populations	microsatellites	?	Not published
Taxidea taxus	Western populations	microsatellites	BC structured, prairies panmictic	Kyle et al. in prep.

Species	**Region (s)**	**Markers**	**Results**	**Authors**
Mephitis mephitis	Tennesse	Allozymes	Genetic variation assessment	Bixler 2000
Lontra canadensis	Alaska	microsatellites	Structured	Blundell et al. 2002
Felidae				
Felis concolor	S. North America	microsatellites	mixed	Walker et al. 2000; Culver et al. 2000; Ernest et al. 2000.
Lynx canadensis	Western North America	microsatellites	panmictic	Schwartz et al. 2002
Lynx rufus	-	-	-	-
Procyonidae				
Procyon lotor	-	-	-	-
Ursidae				
Ursus americanus	Canada	microsatellites	Little structure except to NFLD	Paetkau et al. 1994
Ursus arctos	North America	Microsatellites	Structured	Paetkau et al. 1997, 1998
Ursus maritimus	Circumpolar range	microsatellites	Structured	Paetkau et al. 1995, 1999.
Canidae				
Canis latrans	North America	microsatellites	Little structure	Roy et al. 1994
Canis lupus	North America	microsatellites	Structured	Roy et al. 1994; Forbes and Boyd 1997; Carmicheal et al. 2001; Wilson et al. 2000
Alopex lagopus	Greenland	microsatellites	Structured	Meinke et al. 2001
Vulpes vulpes	-	-	-	-
Vulpes velox	-	-	-	-

Species	Region (s)	Markers	Results	Authors
Urocyon littoralis	Channel Islands and Santa Cruz (CA)	microsatellites	structured	Goldstein et al. 1999; Roemer et al. 2001

Figure 8-1. Relationship of geographic to genetic distance for mustelids.

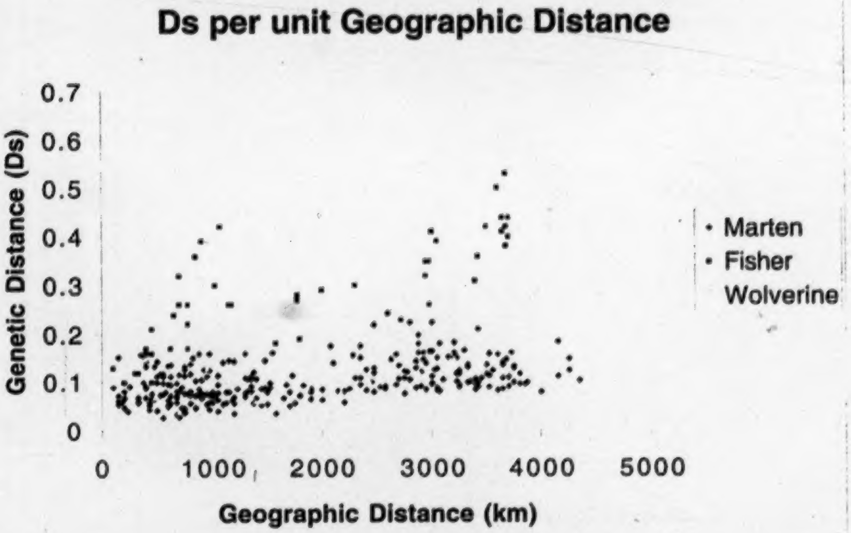

Figure 8-2. Relationship of geographic to genetic distance for other mid-large sized carnivores.